ANNUAL REPORTS IN MEDICINAL CHEMISTRY, 1969

Sponsored by the Division of Medicinal Chemistry of the American Chemical Society

Editor-in-Chief: CORNELIUS H. GAAR
[illegible]

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Sponsored by the Division of Medicinal Chemistry of the American Chemical Society

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PREFACE

This present annual volume is the fifth of a series. Traditionally, a fifth anniversary is a time for retrospection. Readers familiar with the series from its beginning may find it instructive to compare this volume with the first. Many changes will be apparent, and these changes reflect progress in Medicinal Chemistry - greater sophistication in theories, in synthesis, in the approach to new and old problems, in interpretation of subtle differences in biological effects, etc. Equal or greater progress is expected the next five years.

Thanks due the contributors to each volume - authors, section editors and many others - are more deserved each year. There is more to sift and condense, and the material is rarely, if ever, simpler. Their efforts are greatly appreciated.

Fort Washington, Pennsylvania
June, 1970

Cornelius K. Cain

AWARD ADDRESS

Excursions in Medicinal Chemistry - Renal Agents

James M. Sprague
Merck Sharp & Dohme Research Laboratories, West Point, Pa.

Third Award in Medicinal Chemistry, Twelfth National Medicinal Chemistry Symposium of the American Chemical Society, Seattle, Washington, June 22-25, 1970

Medicinal chemists are concerned with the discovery of new therapeutic agents, with the structural design of biologically-active substances and with a chemical interpretation of how these substances bring about their biological effects. Some results of our group in these areas of concern are presented here. Of the several areas that might be selected, the discussion is limited to specific topics in the renal field.

The rapid rate of discovery of highly effective drugs in all fields of therapy over the past twenty to thirty years has been the subject of much discussion. In the renal field, particularly diuretic agents, the notable developments have occurred mainly in the last fifteen years, starting with the first of the sulfonamide type drugs. Prior to this period, the organomercurial diuretics had been the only effective agents since their discovery in 1920. The progress in this area has closed the gap between the level of desirable therapy and the level of therapy attainable prior to these recent developments. Among the newly developed drugs, we have achieved many desirable attributes. Nevertheless, in spite of these advancements, there still exist gaps to be filled before we reach our goal of the elusive, totally satisfactory drug. So far, all of the highly effective drugs cause an undesirable excretion of potassium in the urine that can result in hypokalemia. They also decrease urinary uric acid excretion and increase blood uric acid concentration, a hyperuricemia that has led to gout in predisposed individuals. Many of these drugs can disturb carbohydrate metabolism leading to increased blood glucose levels. While these shortcomings do not occur in all patients and frequently can be overcome by judicious handling of the dose regime and by adjuvant medication, they can and do limit drug use.

These, then, are the major deficiencies to be filled by the design of new diuretics, and they constitute the immediate objectives of many medicinal chemists working in the area of renal drugs. The following discussion concerns some selected aspects of our own work that has been directed to the search for compounds to fill these deficiencies. This discussion will deal with three categories of compounds: (1) compounds designed to mimic the mercurials, (2) the sulfonamides, particularly the hydrothiazides and (3) a heterocyclic class of potassium-sparing diuretics. A complete or detailed analysis of structure-activity relations will not be presented; these either have been or will be published. Emphasis is placed on the inception of these compounds and on certain points related

to a chemical interpretation of how compounds of these classes may exert their biological activity.

Soon after the introduction of organomercurials as diuretics, the idea arose that their biological action resulted from the reaction with and consequent blockade of essential sulfhydryl groups. This was the prevailing concept in 1948-1951 when various of our attempts to design non-mercurial diuretics began. After extensive and sophisticated investigations that have been reported from many laboratories, this concept is still held today. In a recent review, Cafruny states that "the mechanism of action probably involves a firm attachment of mercury to a sulfhydryl group of a renal enzyme that helps to generate energy for sodium transport or to a sodium carrier".

Even today, after the development of many powerful and useful new drugs, the organomercurials are unique among these potent diuretics, in spite of their several disadvantages. The mercurials cause little or minimal potassium loss and, in fact, under certain conditions, may exhibit a potassium-sparing action. They are not known to disturb carbohydrate metabolism. They do not cause uric acid retention and certain of them have been shown to have a uricosuric action in man. Whether these desirable properties are a consequence of their structure and mode of action or result from the intermittent or spaced manner of administration is not clear. But these properties of the mercurials continue to be a challenge to the medicinal chemist and present objectives for the design of new structures.

From these considerations, we have developed in our laboratory several series of highly active compounds that were designed to react selectively with functionally-important sulfhydryl groups, or possibly other nucleophilic groups, that are essential for sodium transport. These compounds generally contain an activated double bond attached to a carboxylic acid structure of a type expected to assist transport into, or excretion by, the kidney. Figure 1 shows a general structure for these compounds,

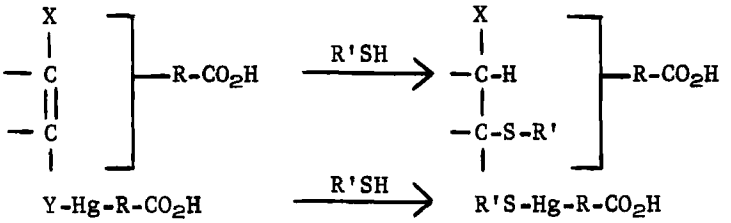


Figure 1

in comparison with a generalized structure for the analogous mercurials, and their reaction with compounds containing SH groups.

Figure 2 gives some of the types of activated ethylenic structures and carboxylic acids employed. All of these ethylene structures have

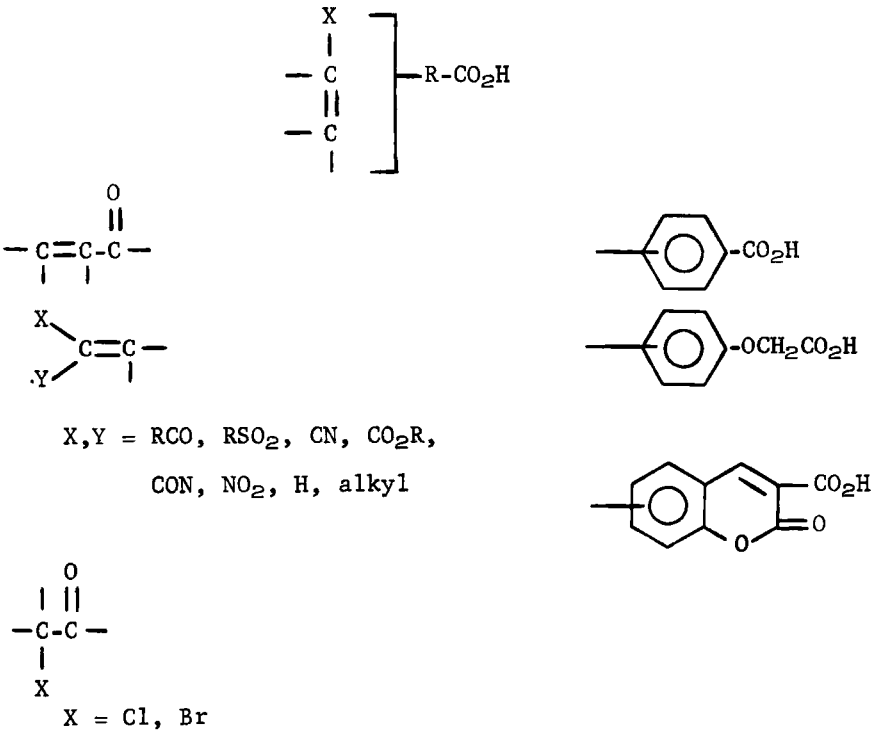


Figure 2

yielded compounds of high activity. The α-haloacyl group has also given active diuretics. Among the carboxylic acid structures, the phenoxyacetic acids are particularly active and have received most attention.

The α,β-unsaturated-acyl compounds (Figure 3) were the first studied,

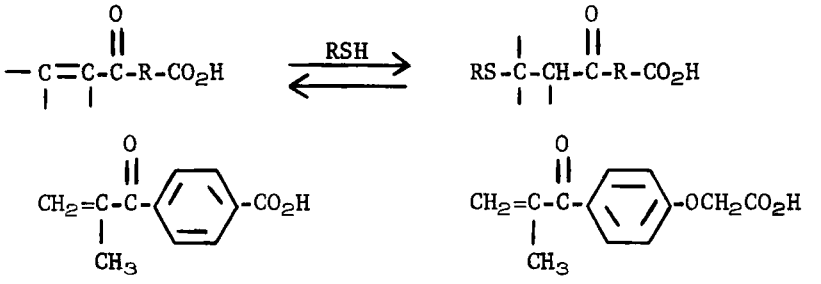


Figure 3

but the initial compounds showed only marginal activity in dogs. At this point in our program, the investigation of these structures was temporarily interrupted by the more promising results arising from the simultaneous study of the aromatic-disulfonamide series that led to the thiazides. In these sulfonamide series, it was clearly demonstrated that an

"activating" group such as halogen, trifluoromethyl, alkyl, etc., adjacent to a sulfamoyl group was required for a useful order of activity. When the work on the unsaturated-acylphenoxyacetic acids was resumed, the effect of the chloro substituent in this series was explored (Figure 4).

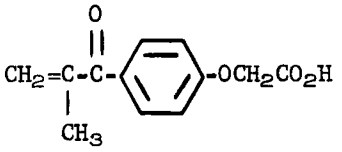
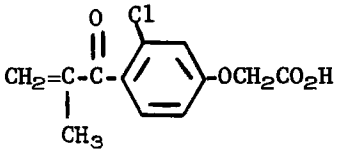
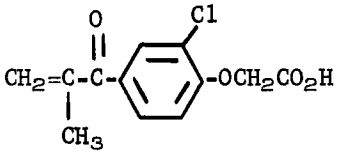
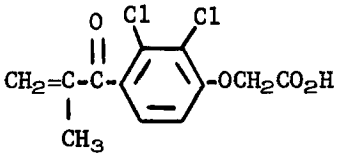
	Diuretic Activity	T 1/2 (min.)
	±	90
	+2	11
	+1	27
	+3	1

Figure 4

Here too, as in the sulfonamides, a tremendous boost in activity was observed, particularly when the chlorine occupied a position ortho to the carbonyl group of the acyl side chain. Not only was biological activity increased but also the rate of the chemical addition of sulfhydryl compounds across the double bond in a model in vitro system. The presence of two chlorines, in positions2 and 3 of the phenoxyacetic acid, further increased both types of activity. The extension of these observations led to several active diuretic series. Representative compounds are given in Figure 5. The first compound is ethacrynic acid, which has been studied extensively, and is highly effective in animals and in man. In the figures, the relative diuretic activity score is based on sodium excretion in dogs following an intravenous administration of a standard dose and the chemical reactivity toward mercaptoacetic acid, as a model sulfhydryl compound, is recorded as the time in minutes for half reaction at pH 7.4 (T 1/2).

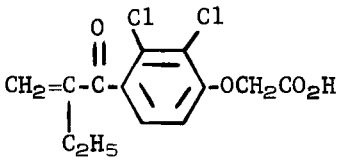
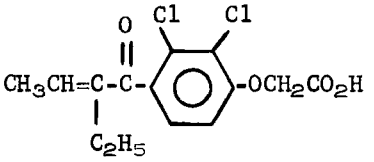
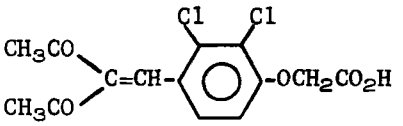
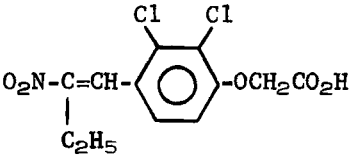
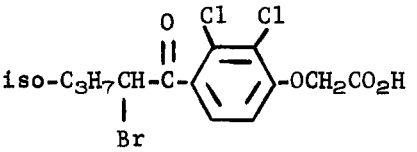
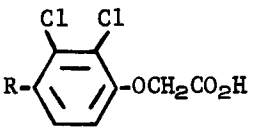
		Diuretic Activity	T 1/2 (min.)
(1)		+6	< 1
	Ethacrynic Acid		
(2)		+5	(210)
(3)		(+7)	2
(4)		+6	2
(5)		+5	(41)

Figure 5

The importance of the presence and position of unsaturation and of halogen in the acyl group is seen in Figure 6. Notable is the weak but real activity of the saturated compound (compound 1). The activity of this compound becomes unequivocal at a dose higher than the standard dose used here for comparison. The introduction of the double bond into the acyl group raises diuretic activity, particularly when it occupies the terminal position (compound 2, ethacrynic acid) which also gives the highest reactivity toward sulfhydryl in vitro. The α-bromo substituent (compound 4) also increased activity; however, not to the level of the unsaturated compounds. But when the bromo substituent is attached to a secondary carbon of the acyl group, as in compound 5, still higher activity results.



	R	Diuretic Activity	T 1/2 (min.)
(1)	$CH_3-CH(C_2H_5)-C(=O)-$	±	N.R.
(2)	$CH_2=C(C_2H_5)-C(=O)-$	+6	< 1
(3)	$CH_3-C(=CHCH_3)-C(=O)-$	+2	(97)
(4)	$CH_3-C(Br)(C_2H_5)-C(=O)-$	+	21
(5)	$C_2H_5-CH(Br)-C(=O)-$	+5	9

Figure 6

Figure 7 shows the point of nucleophilic attack in the three series where a high level of diuretic activity is found. From these results, one may conclude that the phenoxyacetic acid structure, properly substituted, possesses inherent diuretic activity which is accentuated either by unsaturation or by an α-halogen substituent that is capable of attack by a nucleophilic group such as a sulfhydryl. When comparison of diuretic and natriuretic activity is made on the basis of the dose size for given response in dogs, the activated-vinylphenoxyacetic acids (Figure 7, type 1) proved to be most active, for example, the diacetylvinyl-dichlorophenoxyacetic acid (compound 3, Figure 5).

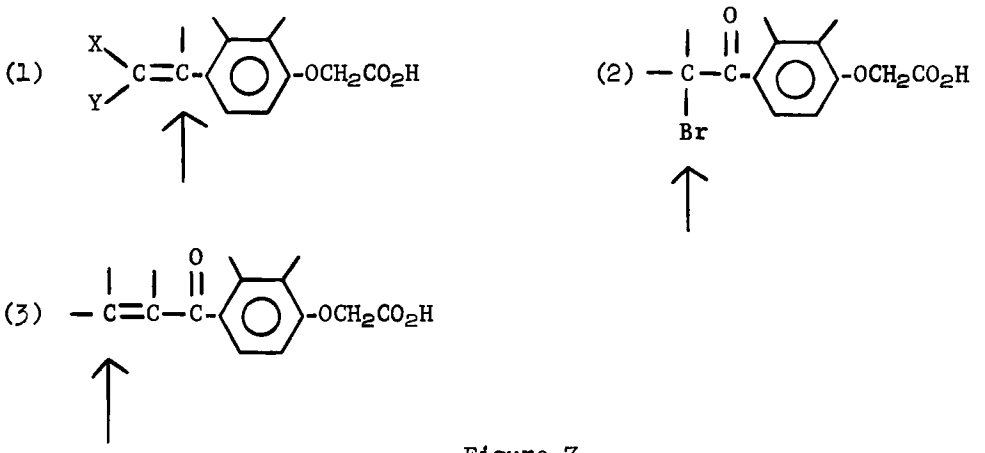


Figure 7

This summary of our results brings us to the question: What is the evidence that the diuretic activity of these mercury-free structures is related to the reaction with a sulfhydryl system or related nucleophilic system? The evidence is mostly circumstantial and must be drawn largely from the extensive studies with ethacrynic acid.

(1) As already noted, maximum diuretic activity is observed only with those structures that are capable of reaction with sulfhydryl.

(2) Ethacrynic acid decreases the protein bound sulfhydryl of the kidney in a manner similar to the mercurials.

(3) Ethacrynic acid reduces the binding of the mercury by kidney tissue.

(4) Ethacrynic acid is excreted in the urine, in large part, as the cysteine adduct in a manner similar to the excretion of mercurials as the cysteine conjugates.

(5) Only those sulfhydryl (or other nucleophilic) adducts of ethacrynic acid are diuretic if they also show in vitro a ready exchange with another sulfhydryl reagent.

From his studies with ethacrynic acid, Cafruny concludes that ethacrynic acid does react with protein-bound sulfhydryl of renal cells. He further states "that the data indicate that ethacrynic acid occupies the same "receptors" and may share the same mechanism of action as the mercurials" and that ethacrynic acid "probably blocks reabsorption of sodium in the same way as mercurials and in most respects is the "non-mercurial mercurial" diuretic it was designed to be".

As can be seen from the data in the figures, there is a lack of quantitative correlation between the biological activity and the chemical

reactivity as exhibited toward sulfhydryl compounds *in vitro*. In view of the complex animal system for assaying biological activity and the simple *in vitro* system for measuring chemical reactivity, this lack of correlation is not unexpected. A better correlation may be expected if the chemical system measured the displaceability of one sulfhydryl by another, one reagent chosen to represent the sulfhydryl encountered in transport to the kidney and the other the sulfhydryl that is in the receptor tissue (Figure 8). Preliminary data support this view. For example, in the cysteine adduct of ethacrynic acid, which itself is a highly active diuretic, the cysteine can be displaced by reaction at pH 7.4 with mercaptoacetic acid, thus giving the more stable adduct of mercaptoacetic acid that is inactive as a diuretic.

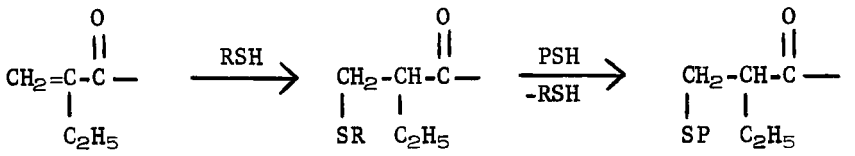


Figure 8

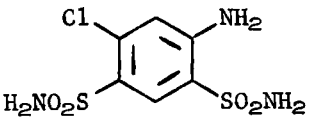
This approach to the design of structures that mimic the diuretic activity of the mercurials has led to the extremely potent compounds that lack certain of the disadvantages of the mercury-containing drugs and may share, in part, a common mechanism of action. However, other desirable attributes of the mercurials have not been reproduced. Ethacrynic acid, the only compound that has had extensive study both in animals and in man, causes potassium loss and uric acid retention and perhaps some disturbance of glucose metabolism although this latter effect appears to be minimal compared to that observed with many diuretics of the sulfonamide class.

To this point in the discussion, biological activity has referred to the response following a standard intravenous or oral dose. For comparison within the series, this is adequate. However, these compounds exhibit a quantitatively different dose response from that of the thiazides. The response to ethacrynic acid is less than for hydrochlorothiazide at the low doses but, at higher doses, the ethacrynic acid response exceeds that obtainable with any dose of chlorothiazide or of any of its relatives. The ethacrynic acid congeners all exhibit a similar dose response curve, showing the higher maximums than the thiazides, and differing within the series only by the position on the dose scale.

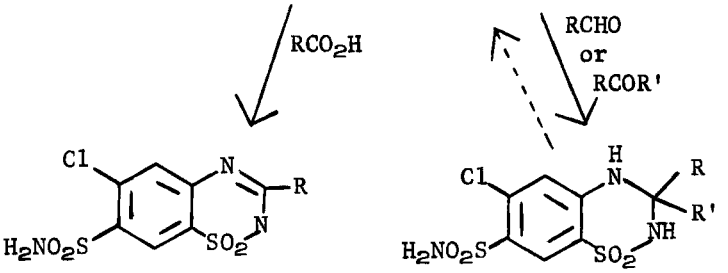
During the course of our work that is described here, a novel sulfonamide diuretic, furosemide, was developed in Germany. This compound (4-chloro-N-(2-furylmethyl)-5-sulfamoylanthranilic acid), a sulfonamide that has appreciable carbonic anhydrase inhibitory activity and shows no reaction toward sulfhydryl compounds, exhibits a dose response that is unique among all known sulfonamide diuretics. The response is similar to that of ethacrynic acid with similar high maximum of natriuresis.

However, it, too, causes potassium loss, uric acid retention and an alteration in glucose metabolism. How two compounds, ethacrynic acid and furosemide, of such diverse structures elicit similar biological responses lacks, so far, a chemical interpretation.

The sulfonamide diuretics all trace their origin to the observation that the inhibition of carbonic anhydrase in the kidney, first by sulfanilamide and, later, by the much more potent acetazolamide, leads to excretion of sodium but very little chloride, and large amounts of potassium and bicarbonate. From this beginning have arisen many sulfonamide diuretics of widely different structures that cause more chloride, less bicarbonate and less potassium excretion than the first carbonic anhydrase inhibitors. As this electrolyte excretion pattern improved with structural modifications, the inhibitory activity toward carbonic anhydrase, as measured in vitro, generally decreased markedly. Our investigation of aromatic-disulfonamides and the related thiazides illustrates this trend. A marked improvement in the electrolyte excretion pattern was noted with 5-chloro-2,4-disulfamoylaniline (CDSA)(Figure 9). Activity



Chlorodisulfamoylaniline (CDSA)



Thiazides Hydrothiazides

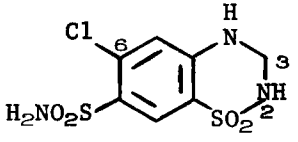
Figure 9

was further improved by cyclization to the thiazides and hydrothiazides. These are saluretic agents causing excretion of approximately equivalent amounts of sodium and chloride, little or no bicarbonate and substantially less potassium than the earlier sulfonamides. These sulfonamides all exhibited, in vitro, considerably weaker inhibition of carbonic anhydrase than acetazolamide; CDSA has approximately 1/60, chlorothiazide 1/40 and the hydrothiazides 1/200 - 1/600 the activity of acetazolamide. Thus, the hydrothiazides possess enzyme inhibitory activity that is only equal to, or even less than, sulfanilamide. Modification of the substituent in the 3-position of the hydrothiazide structure has produced

the greatest increase in diuretic activity. As measured in terms of the amount of compound that is required for a given response, the hydrothiazides range up to 2000 times more active than chlorothiazide. Within the hydrothiazide series, this increased diuretic activity is achieved without a marked change in the relative in vitro enzyme inhibition. These low enzyme inhibitory activities, together with the marked improvement in the electrolyte excretion pattern and the increased potency of the thiazides, generally, raise the question of the relevance of enzyme inhibition to the saluretic activity and to the potassium loss. Nevertheless, the inhibitory activity, although low, is real and all of the compounds contain the unsubstituted-sulfamoyl group (or a group metabolizable to such a group) that has been repeatedly shown to be responsible for enzyme inhibition. Furthermore, over the years, reports have appeared dealing with the action of the sulfonamide (including the thiazide) diuretics on many enzyme systems, but carbonic anhydrase remains the most sensitive to inhibition by these compounds.

In searching for a rationalization of these structural influences and for a chemical basis for the design of compounds to surmount the deficiencies, particularly potassium loss, a number of chemical and physical parameters has been considered. Two, lipid solubility and chemical stability, have yielded provocative results (Table I). In the hydrothiazide series, the substituents in the 3-position that so greatly increase diuretic activity also markedly increase lipid solubility. One may consider that this property relates to the absorption and/or transport of the compound to the site of action. Chemical stability measurements in aqueous buffered solutions in the pH range 4-8 at 37° indicate that these structural changes also lead to increased chemical instability and to hydrolysis to the CDSA from which the compound was originally prepared by cyclization (Figure 9). Pertinent to these considerations are two properties of CDSA. It is less active as a diuretic than chlorothiazide in the dog, possibly only 1/2 as active, but, in man and in rats, it is 2-3 times as active. Also, as a carbonic anhydrase inhibitor in vitro, it is more active than the hydrothiazides and approximates the activity of chlorothiazide.

However, the chemical stability of two highly active compounds of this series presents exceptions. Polythiazide and methyclothiazide (compounds 4 and 5, Table I) having methyl substituents on the nitrogen in the 2-position exhibited little or no cleavage in vitro to the corresponding CDSA derivative. But, for one of these compounds, polythiazide, a major excretion product in the urine is reported to be the cleaved product, the methyl-CDSA derivative. For the other hydrothiazides, no reliable quantitative data are available on the nature of the form excreted in the urine although detectable cleavage is reported. These results suggest the intervention of in vivo processes and that metabolic cleavage of the benzothiadiazine ring may occur. The incubation of representative hydrothiazides with liver or kidney homogenates in vitro, however, did not show increased cleavage over that observed in the buffer chemical system.



	3-Substituent	Relative Diuretic Activity Dog	Man	Lipid Partition[1]	Relative Carbonic Anhydrase Inhibition[2]	Hydrolysis % 24 hrs., 37° pH 4	pH 8
(1)	H	3+	10	1.0	0.4	1	13
(2)	H (6-CF_3)	3+	10	0.8	0.03	8	6
(3)	$C_6H_5CH_2$-(6-CF_3)	4+	100-200	15	0.07	18	68
(4)	CF_3CH_2S-CH_2-(2-CH_3)	5+	500	14	1.3	(<5)	0
(5)	$ClCH_2$-(2-CH_3)	4+	200	26	1.6	(<1)	0
(6)	Cl_2CH-	5+	100-200	4	0.3	45	5
(7)	5-norbornen-2-yl	4+	250	63	0.3	--	--
(8)	cyclopentyl-CH_2	5+	1000-2000	19	0.6	37	92
(9)	4'-methyl-spirohexane	5+	100	7	0.7	85	78
	Chlorothiazide	2+	1.0	0.3	5	< 1.0	< 1.0
	CDSA	(+)	2-3	--	3.4	--	--

[1] octanol - pH 6.5 buffer

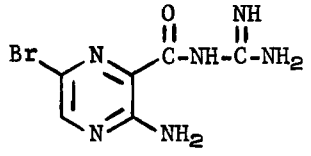
[2] sulfanilamide = 1.0

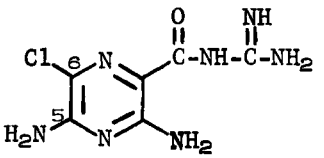
Table I

Whether the increased tendency of these more highly active agents in the hydrothiazide series to revert to the corresponding CDSA in chemical or biological systems has any significance for an interpretation of the mechanism of the natriuretic or kaluretic action or of the common natriuretic ceiling response requires more study. In any event, such considerations can apply only to the hydrothiazides and not to the thiazides, which are relatively more stable, or to many other types of sulfonamide diuretics that cannot yield a CDSA-like product.

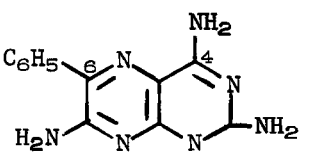
In connection with the uric acid retaining property of diuretics, it is significant to note that several of the thiazides and also ethacrynic acid, when administered intravenously, first elicit a uricosuric response of brief duration followed by uric acid retention. However, after oral administration, only the retention is observed. This two-phase response is presumably related to the blood concentration of the drugs presented to the kidney. It will be recalled that certain uricosuric drugs that are not natriuretic, particularly probenecid and salicylic acid, at low levels, show a uric acid retention while higher levels produce uric acid excretion. Further, certain compounds, particularly of the pyrazolidinedione series, have some actions that are the opposite of the diuretics. An example is sulfinpyrazone which causes an increase in uric acid excretion but can also decrease sodium excretion. Whether there is a clue here for the design of better diuretics has not been determined.

In seeking compounds that might have no or minimal kaluretic effect, we were forced to return to a more empirical approach. A lead was sought by screening procedures, and one was turned up in (6-bromopyrazinoyl)-guanidine, a compound available to us through previous work in our





Amiloride



Triamterene

laboratories concerned with syntheses in the folic acid series. The introduction of an amino (or substituted-amino) group in the 5-position markedly improved the sodium and chloride excretion activity without effecting potassium excretion. (5-Amino-6-chloropyrazinoyl)guanidine (amiloride) was among the most promising in animals and in man. This compound increases sodium and chloride excretion with no concomitant increase in potassium output. Its saluretic activity is, however, less than for other diuretics. But when co-administered with the kaluretic diuretics, such as acetazolamide, the thiazides or ethacrynic acid, there is an enhanced natriuresis with conservation of potassium.

During the course of these studies, reports from other laboratories indicated similar properties for pteridine derivatives. Triamterene,

2,4,7-triamino-6-phenylpteridine, has become a successful drug. Both amiloride and triamterene are potassium-sparing diuretics that exhibit only moderate saluretic activity. Both will reverse the sodium retention and potassium excretion action of aldosterone, but they are not aldosterone antagonists since they are active in the absence of the hormone.

Although the pyrazinoylguanidines and the pteridine diuretics were developed simultaneously and independently, the related nature of their structures and their similar biological actions has raised the question of whether the pteridines are, in fact, "ring closed" versions of the pyrazinoylguanidines or the pyrazinoylguanidines are "ring opened" versions of the pteridines. While this question cannot be answered unequivocally, a consideration of some of the chemical and biological characteristics and the influence of the effects of structural changes in the two series indicates that this resemblance may be more apparent than real.

Among the useful diuretics, the unique characteristic of amiloride and related pyrazinoylguanidines is the strong basic property. Other diuretics are acids or weak bases. Amiloride has a pK_a of approximately 8.7. This strong basic character is attributed to the guanidine structure. Triamterene is a weak base with a pK_a of 6.2.

In both series, an "activating" group in the 6-position is essential for a useful order of activity. In the pteridines, either chlorine or phenyl substitution produces high activity. However, only chlorine is effective in the pyrazinoylguanidines while the phenyl derivative is essentially inactive. Replacement of the 4-amino of the triamterene structure with hydroxyl destroys activity while the carbonyl oxygen of the corresponding position (α) in the "opened" analog in the pyrazinoylguanidine series is required, and its replacement by an imino group greatly reduces activity. These and other structural comparisons suggest that each series is distinctly different. Because of technical difficulties, studies dealing with the biological interaction when amiloride and triamterene are administered together have not yielded clear-cut, useful data related to these structural questions.

While these two classes of diuretics have effectively overcome the kaluretic problem, and perhaps the disturbance of uric acid and glucose metabolism, it has been accomplished at the price of a greatly decreased saluretic activity, the activity that is the basis of diuretic agents.

Appreciation and acknowledgment - I deeply appreciate the honor of being the recipient of the Third Medicinal Chemistry Award and offer my thanks to the Medicinal Chemistry Division. Also, I want to acknowledge my indebtedness to my many other associates, chemists, biologists and clinicians, who, over the past thirty years, have made my efforts possible. I do not pretend to have drawn exclusively from my own resources. The number of these associates is too long to list here; I trust that they each recognize their contributions to our joint efforts and accept my thanks and appreciation.

Section I - CNS Agents

Editor: Scott J. Childress,
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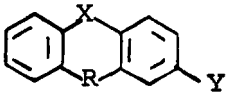
Chapter 1. Antipsychotic and Anti-anxiety Agents

R. Ian Fryer, Hoffmann-La Roche Inc., Nutley, New Jersey

Introduction - Several new antipsychotic or anti-anxiety drugs were marketed in Europe during the last year, while in the United States, only one compound (doxepin) reached the physicians armamentarium. While a great effort in the molecular modification of well-defined classes of active compounds is still being carried out, a number of new structures have been reported (either pharmacologically or clinically) to show promise as future drugs in the CNS area. As in previous years, a large amount of work has been reported, trying to elucidate the mechanism of neurotic and psychotic disorders.

Tricyclic Compounds with 6-membered rings -



Ia-e

a) X = S ; Y = CN; R = $CHCH_2CH_2N$⟨⟩$-OH$

b) X = S ; Y = H ; R = $NCH_2CH(CH_3)CH_2N$⟨⟩$NCH_2CH_2OCH_2CH_2OH$

c) X = NH ; Y = Cl; R = $CH(CH_2)_3N(CH_3)_2$

d) X = S ; Y = Cl; R = $C{=}CH(CH_2)_2N$⟨⟩NCH_2CH_2OH

e) X = S ; Y = $S\overset{\downarrow}{O}CH_3$; R = $N(CH_2)_2$-⟨N⟩-$\underset{\downarrow}{CH_3}$

Ten medical teams reported on the effectiveness of pericyazine (Ia) in 269 patients with major psychotic disturbances.[1] The drug was considered as a potent agent for reducing agressivity and character disturbances. Although dixyrazine, Ib, was reported as being less active than chlorpromazine in its depressive action on the CNS, its antihistaminic and antiemetic properties were much stronger. One advantage

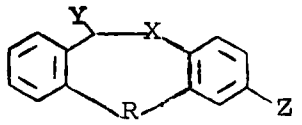
reported was its weaker effect on the blood pressure.[2] Another clinical study reported that the drug was very useful in depression, anxiety and irritability.[3]

The antipsychotic efficacy and low toxicity combined with fewer and less severe side effects than "prototype" antipsychotics were reported by Bishop and co-workers[4] as advantages for the use of clomacran (SKF 14336) (Ic). Additional clinical trials have been carried out on Ic in order to determine the effectiveness of the drug in psychoses.[5,6] Clopenthixol (Id), a xanthene analogue of perphenazine, was shown in several studies to be an effective antipsychotic.[7,8] Metabolic studies were carried out in rats and man,[9] and unlike the phenothiazines, no phenolic metabolites were detected. Clopenthixol is excreted as the glucuronide together with three other metabolites. One of these is the sulfoxide while the other two involve loss of $-CH_2CH_2OH$ from clopenthixol and its sulfoxide. Mesoridizine (Ie) was first reported as a metabolite of thioridazine. A double blind study[10] shows efficacy in schizophrenic patients equal to chlorpromazine but at 1/2 the dose levels. This compound was also rated better than chlorpromazine in studies on children with behavioral problems.[11] Mesoridizine was approved for marketing in the U.S., (February, 1970).

The observation that mono-hydroxy derivatives of chlorpromazine are further hydroxylated in *in vitro* systems led to the preparation of two new derivatives of chlorpromazine.[12] The 7,8-dimethoxy and 7,8-dimethylmethylenedioxy compounds were prepared and underwent extensive pharmacological evaluation. Both were less active than chlorpromazine in mice and rat studies.[12]

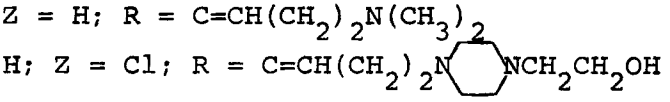
Tricyclic Compounds with a Seven membered ring -

Doxepin (IIa) was marketed this year as a mixture of *cis* and *trans* isomers (acid catalyzed equilibration in the final step of the synthesis gave a *cis* to *trans* ratio of approximately 15:85).[13] This drug was compared with amitriptyline[14] and found to be clinically similar in its properties. Side effects were due to its anticholinergic properties. Several clinical studies with doxepin showed that the drug was a good antidepressant. One report stated that there was noticeable sedation[15] while others claimed only mild side effects.[16]



IIa-d

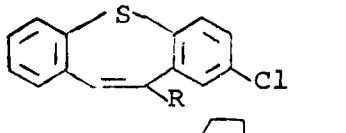
a) X = O ; Y = Z = H; R = $C{=}CH(CH_2)_2N(CH_3)_2$

b) X = O ; Y = H; Z = Cl; R = 

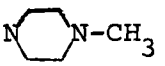
c) X = SO_2; Y = Z = H; R = $CH(CH_2)_3N(CH_3)_2$

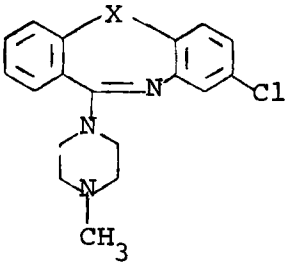
d) X = SO_2; Y = $(CH_2)_2N(CH_3)_2$; Z = H; R = CO

Metabolic studies[13] were carried out in rats and dogs and the major metabolic pathway appeared to be that of phenolic hydroxylation and N-dealkylation. Glucuronide and N-oxide formation was also observed. The related compound, pinoxepine (P-5227), IIb, was reported to have a greater antipsychotic effect than perphenazine in human studies.[17]

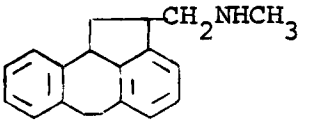


IIIa, R =

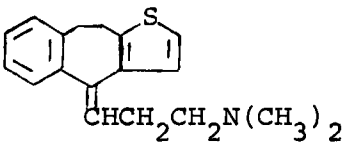
IIIb, R = 



IVa, X = O
IVb, X = S



V



VI

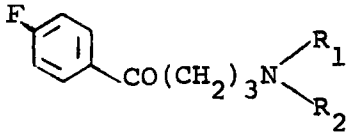
The preparation of compounds of types IIc and IId were reported by Protiva and co-workers.[18] These compounds had little or no CNS activity. Dibenzothiepin derivatives, IIIa, b, were equal to perphenazine in mice and rat studies.[19]

Additional neuropharmacological studies on the potent, related oxilapine (dibenzoxazepine), compound IVa have been reported.[20] A series of articles on the animal and human metabolism of the related clotiapine (IVb) appeared this year.[21-23]

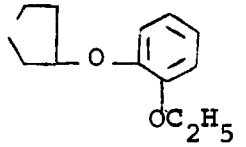
The synthesis of so-called "bridged" amitriptyline analogues (e.g., V) was reported.[24] Animal studies showed no antidepressant properties but some of the compounds did exhibit CNS depressant activity. A two year clinical study of IB 503, compound VI, as a mild tranquilizer reportedly gave

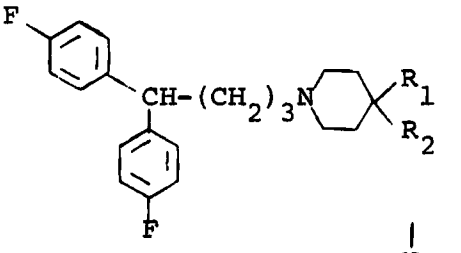
very good results in about 80% of patients.[25]

Compounds Related to Butyrophenones -

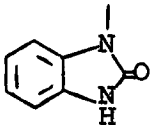


VII R_1R_2 =

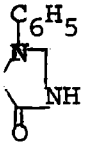




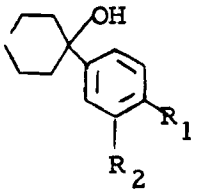
IXa,b a) R_1 = H; R_2 =



b) R_1R_2 =



VIIIa-d R_1R_2 =



a) R_1 = Cl; R_2 = H

b) R_1 = H; R_2 = CF_3

c) R_1 = CH_3; R_2 = H

d) R_1 = Cl; R_2 = CF_3

Pyrrolidine derivatives of butyrophenones have been synthesized and evaluated pharmacologically.[26] Compound VII was reported to have three times the activity of haloperidol in mice. Hypotensive effects in dogs were also noted. By studying the distribution of haloperidol (VIIIa), trifluperidol (VIIIb), moperone (VIIIc) and clofluperol (VIIId) in the brain, blood and liver of the rat, an attempt was made to correlate neuroleptic activity with drug brain levels.[27] A similar study, examining protein binding and the lipophilic nature of drugs in the carbamate and benzodiazepine groups has also been reported.[28] Omission of the 4-phenylpiperidyl moiety in VIII combined with the formation of a carbamate ester was reported to decrease activity in cats (<haloperidol).[29] Replacement of the keto group by other functions also led to compounds with central depressant-tranquilizer properties in mice.[30] Studies[31] in rats carried out on the long acting and potent neuroleptic drug, pimozide IXa, have shown that the major metabolic pathway is oxidative N-dealkylation.

The new neuroleptic (which also demonstrates that the keto group is not essential for activity) fluspirilene (IXb) has been shown to be an extremely potent and long acting drug

in the clinic.[32] Metabolic studies again indicate that the major pathway is oxidative N-dealkylation.[33]

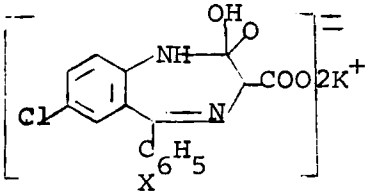
Carbamates - A series of 2-substituted 1,3-propanediol dicarbamates were prepared and the pharmacology of these compounds together with structure-activity relationships were discussed.[34]

A study of the kinetics of distribution and metabolism of meprobamate, carisoprodol and tybamate was made using mice.[35] The author was able to correlate higher brain levels of the drugs with increased lipid solubility (see also reference 28).

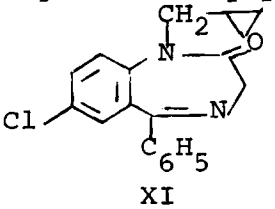
Benzodiazepines - Chlorazepate (X) is now marketed in France as a tranquilizer with a benzodiazepine profile. The synthesis of this and related compounds together with a discussion of pharmacologic activity in animals has been reported.[36] The study of the metabolism of prazepam (XI) in dogs showed the typical products of dealkylation and 3-hydroxylation.[37] Medazepam (XII) recently introduced on the market in Europe was the subject of numerous clinical and pharmacological articles. The metabolism and pharmokinetics of this drug were also extensively investigated.[38] The synthesis of oxazolazepam (XIII) was reported[39] as was the acute and chronic toxicity data in animals.[40]

The synthesis of other types of benzodiazepine derivatives was also reported.[41-45] In some cases, pharmacological results were given.[43-45]

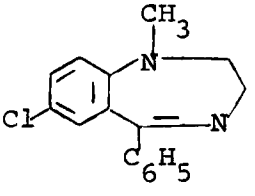
Temazepam (XIV) was reported to have marked and prolonged anticonvulsant effects in rabbits[46] and also to be of value in the treatment of insomniac patients with neurosis or endogenous depression.[47] Nitrazepam (XVa) was reported as a useful agent in the treatment of children with resistant myclonic seizures.[48] The utility of clonazepam (XVb) as an anticonvulsant agent was the subject of a symposium.[49]



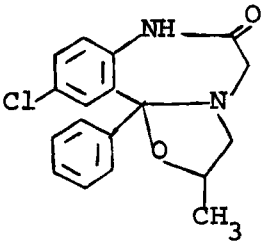
X



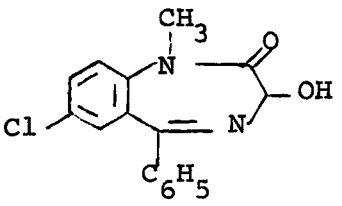
XI



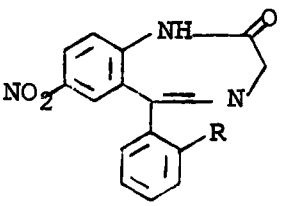
XII



XIII

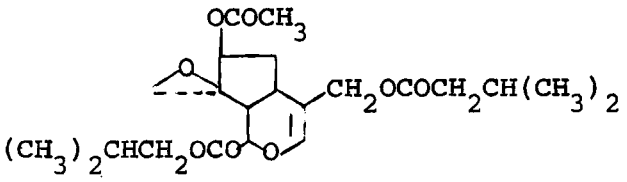


XIV

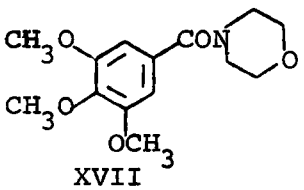


XVa: R = H
XVb: R = Cl

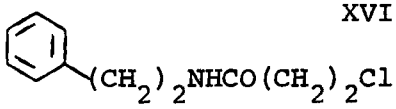
Structures of Current Interest -



XVI



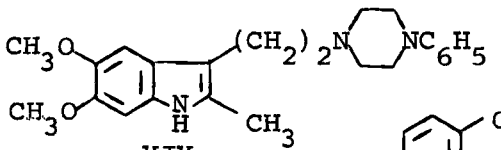
XVII



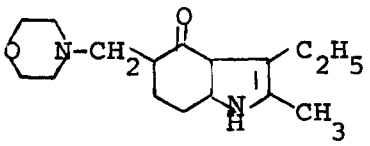
XVIII

A mixture of closely related natural substances, isolated from the roots of species of Valerina and Kentranthus, was shown to have tranquilizing properties in cats and mice.[50] The major constituent (80%) is didrovaldratrum (XVI). Clinically, this mixture has shown properties of a minor tranquilizer without sedative effects.[51] It has no antipsychotic activity. Trioxazine (XVII) previously reported as a mild tranquilizer was shown to be effective in the treatment of nervous disorders in children.[52]

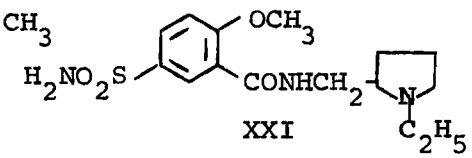
Additional studies on phenacon (XVIII) indicate that besides its anti-epileptic properties, this compound possesses the characteristic properties of the minor tranquilizers.[53]



XIX



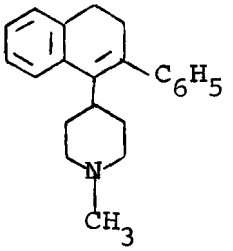
XX



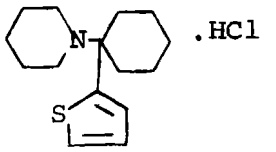
XXI

Additional studies on oxypertine (XIX) indicate that the major advantage of this compound is its mood elevating and stimulant properties in schizophrenics where apathy is a major problem.[54] In a double blind study with chlorpromazine oxypertine was judged to be of value in the treatment of chronic schizophrenia.[55] In two clinical studies, molindone (XX) was shown to be equal to trifluoperazine as an antipsychotic but antidepressant properties were not noted at the dosage used.[56,57]

Sulpiride, XXI, has been introduced in Europe and has been reported to be a clinically important new class of neuroleptic agent[58] with strong anti-emetic properties.[59] In mice no anticonvulsant activity was noted.[60]

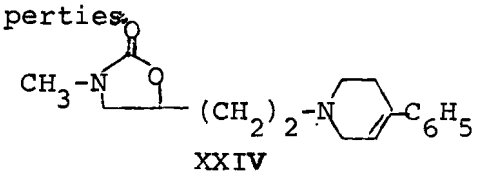


XXII

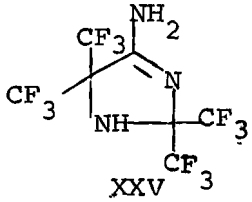


XXIII

A new psychosedative HS-2314 (XXII) was compared with and differentiated from the benzodiazepines and phenothiazeines in cats and rabbits.[61] A series of N,N-substituted 1-arylcyclohexylamines were prepared and screened for their psychopharmacological properties.[62] The thienyl derivative, XXIII, was the most active in terms of sedative, taming and ataxia properties.



XXIV



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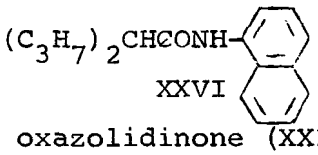
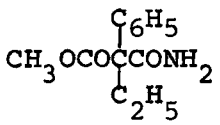


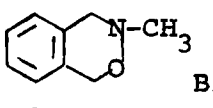
XXVI

A substituted oxazolidinone (XXIV) which had the pharmacological spectrum of a major tranquilizer was reported in a clinical study to worsen psychosis and no beneficial effect was noted.[63] The unusual, fluorinated aminoimidazoline (XXV) has been reported to have the pharmacological properties of a minor tranquilizer.[64]

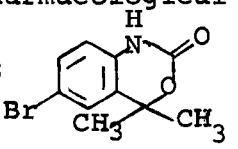
Six amides derived from dipropylacetic acid (e.g. XXVI) were reported to have tranquilizing properties in mice and are scheduled for extensive pharmacological evaluation.[65]



XXVII



XXVIII



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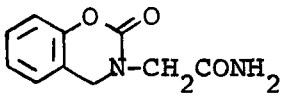
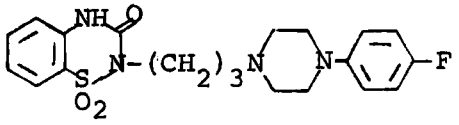


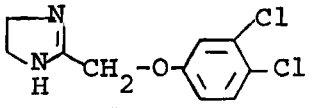
XXX

Methyl ethylphenylmalonamate (XXVII) was reported to have a meprobamate like profile in animals.[66] A series of 2,3-benzoxazines related to XXVIII were prepared and studied pharmacologically.[67] All compounds exhibited a less pronounced CNS depressant effect than did the earlier reported compound, XXVIII.

The 3,1-benzoxazine (XXIX) was reported as the most active of a series of CNS depressants in mice.[68] These benzoxazinones[69] did not show the antidepressant properties of F.I. 6654 (XXX).



XXXI



XXXII

The activity of a benzothiadiazine (XXXI) was reported as comparable to chlorpromazine in animal experiments.[70] Initial clinical trials indicated promise that the imidazoline XXXII might be a worthwhile antidepressant.[71] A review on the therapeutic use of lithium carbonate in the treatment of mania, excitement, epilepsy and premenstral tension has been given by Noyes.[72] This compound has just been approved for introduction on the U.S. market (April, 1970).

Pharmacological Investigations - Pitts and McClure[73] reported that patients with anxiety neurosis show increased levels of lactate in the blood when under conditions of physiological stress or physical exercise. Infusion of lactate in susceptible patients in a double blind study initiated anxiety symptoms. A proposal that over-production of adrenalin by these patients is the cause for increase of lactate was supported by reports that the β-blocker, propranolol, can reduce or eliminate anxiety symptoms. Grosz and Farmer[74] in a critical evaluation of Pitts and McClure's work, feel that these authors

have not experimentally demonstrated their hypothesis.

In a series of three articles, Schildkraut[75] gives an excellent review of diagnoses, the use of and the neuropsychopharmacology of various types of drugs and biogenic amine metabolism in patients with affective disorders. Loew and Taeschler[76] examined profiles of pharmacological activity as a means to predict the therapeutic effects of psychotropic drugs.

p-Chlorophenylalanine (P-CPA) is a selective serotonin depletor reported to deplete brain serotonin by about 90%. Robichaud and Sledge[77] try to correlate the role of serotonin in the brain and its role in mental disease. In animal avoidance tests, they demonstrated an effect qualitatively similar to those reported for anti-anxiety agents such as meprobamate, chlordiazepoxide and reserpine. The effects were demonstrable 30 minutes after oral administration and lasted as long as 6 days. P-CPA works by inhibition of tryptophan hydroxylase. The compound also is an inhibitor of phenylalanine hydroxylase *in vitro* and *in vivo*. Watts and Martin[78] demonstrated a serious defect in the acquisition of learning in the swimming maze by injecting P-CPA in young rats for approximately 30 days. Toxicity was evidenced in terms of cataracts, eczema and loss of brain and body weight.

A recent World Health Organization report, summarized in Nature[79] establishes a strong case for linking much mental disease with malnutrition, particularly during early life. While mania and depression are related to the metabolism of the cerebral monoamines and to changes in water balance and sodium ion concentration, no specific biochemical correlation has yet been demonstrated for the schizophrenic's visions and voices. The report suggests that the only rational approach for the biochemist in the study of schizophrenia is drug induced psychosis. Large single doses of LSD have been shown to effect metabolic changes (increase in serotonin and decrease in norepinephrine).

REFERENCES

1. J.Bobon, J.M.Gernay, J.Collard, F.Goffioul and M.Breulet, Acta.Neurol.Belg., 68, 154 (1968).
2. M.D. Mashkovsky and A.I.Polezhaeva, Farmakol.Toksikol., 32, 28 (1969).

3. L.Bodendorfer, Nord.Psykiat.Tidd., 22, 64 (1968).
4. M.D.Bishop, L.B.Mason, D.M.Gallant and G.Bishop, Curr. Therap.Res., 11, 447 (1969).
5. B.J.Goldstein and S.Jacobson, Dis.Nerv.Sys., 30, 37 (1969).
6. W.T.Lampe, Curr.Therap.Res., 11, 300 (1969).
7. A.Hicklin and J.Angst, Schweiz.Med.Wschr., 19, 615 (1967).
8. O.Trotta, Osped.Psichiat., 35, 535 (1967).
9. A.R.Khan, Acta.Pharmacol.et.Toxicol., 27, 202 (1969).
10. H.Freman, M.R.Oktem and N.Oktem., Curr.Therap.Res., 11, 263 (1969).
11. T.J.Kenny, D.Badie and R.W.Baldwin, J.Nerv.and Mental Dis., 147, 316 (1968).
12. A.A.Manian, N.Watzman, M.L.Steenberg and J.P.Buckley, Life Sci., 7, 731 (1968).
13. D.C.Hobbs, Biochemical Pharmacology, 18, 1941 (1969).
14. G.Bauer and H.Nowak, Arzneim.Forsch., 19, 1642 (1969).
15. G.Belsasso, H.L.Topia and A.Grajales, Curr.Therap.Res., 11, 165 (1969).
16. See for example, F.J.Ayd, Dis.Nerv.Sys., 30, 396 (1969).
17. J.M.C.Holden, T.M.Itil and A.Keskiner, J.Clin.Pharmacol. J.New Drugs, 9, 163 (1969).
18. M.Rajsner, E.Svatek, V.Seidlova, E. Adlerova and M.Protiva, Angew.Chem., 81, 1278 (1969).
19. K.Sindelar, J.Metysova, J.Metys and M.Protiva, Naturwissenschaften, 56, 374 (1969).
20. C.N.Latimer, J.Pharmacol.and Exp.Ther., 166, 151 (1969).
21. W.Michaelis, Farmaco.Prac., 26, 84 (1969).
22. R.Gauch, F.Hunziker, W.Michaelis and O.Schindler, Farmaco. Prac., 26, 92 (1969).
23. R.Gauch and H.Lehner, Farmaco.Prac., 26, 100 (1969).
24. E.Galantay, C.Hoffman, N.Paolella, J.Gogerty, L.Iorio, G.Leslie and J.H.Trapold, J.Med.Chem., 12, 444 (1969).
25. E.Messmer, Arzneim.Forsch., 19, 735 (1969).
26. W.J.Welstead, G.C.Helsley, R.L.Duncan, A.D.Cale, C.R.Taylor, J.P.DaVanzo, B.V.Franko, C.D.Lunsford, J.Med.Chem., 12, 435 (1969).
27. P.A.J.Janssen and F.T.N.Allewijn, Arzneim.Forsch., 19, 199 (1969).
28. E.Van Der Kleijn, Arch.int.Pharmacodyn., 179, 225 (1969).
29. R.Guerrero-Figueroa, M.M.Rye, D.M.Gallant and C.L.Morse, Curr.Therap.Res., 11, 121 (1969).
30. R.L.Duncan, G.C.Helsley, W.J.Welstead and B.V.Franko, J.Med.Chem., 12, 442 (1969).
31. W.Soudijn and I.VanWijngaarden, Life Sci., 8, 291 (1969).

32. H.J.Haase, Th.Frank, M.Knaack, Ch.Lehnhardt and H.Richter-Peill, Nervenarzt, 39, 275 (1968).
33. J.P.P.Heykants, Life Sci., 8, 1029 (1969).
34. B.J.Ludwig, L.S.Powell and F.M.Berger, J.Med.Chem., 12, 462 (1969).
35. E.Van Der Kleijn, Arch.Int.Pharmacodyn., 178, 457 (1969).
36. J.Schmitt, P.Comoy, M.Suquet, G.Callet, J.LeMeur, T.Clim, M.Brunaud, J.Mercier, J.Salle and G.Siou, Chim.Ther., 4, 239 (1969).
37. F.J.Dicarlo, M.C.Crew, M.D.Melgar and L.J.Haynes, J.Pharm. Sci., 58, 960 (1969).
38. J.Rieder and G.Rentsch, Arzneim.Forsch., 18, 1545 (1968).
39. Netherlands Patent 16789 (1968) assigned to Sankyo Co. Ltd.
40. H.Masuda, T.Nishiyama, S.Shimada, Y.Inazu, T.Okonogi and Y.Suzuki, Annual Report of Sankyo Research Laboratories 21, 82 (1969).
41. G.Hardtmann and H.Ott, J.Org.Chem., 34, 2244 (1969).
42. H.P.Harter and S.Liisberg, Acta.Chem.Scand., 22, 3332 (1968).
43. M.Descamps, J.Van der Elst, F.Bioron, F.Chaillet, A.Christiaens, Chim.Ther., 3, 260 (1968).
44. F.DeMarchi and M.V.Torrielli, Chim.Ther., 3, 430 (1968).
45. F.DeMarchi, G.F.Tamagnone and M.V.Torrielli, Boll.Chim. Farm., 107, 416 (1968).
46. T.Mille, G.Pastorino and A.Arrigo, Arnzeim.Forsch. 19, 730 (1969).
47. C.Maggini, M.Murri and G.Sacchetti, Arnzeim.Forsch., 19, 1647 (1969).
48. R.Baldian, T.J.Kenny and J.Segal, Curr.Ther.Res., 11, 413 (1969).
49. Intern. Congr. Neurol. Surg., 4th - Intern. Congr. Neurol., 9th, New York, N.Y., Sept.20-27, 1969. Intern. Congr. Ser. No. 193. Excerpta Med. Found., Amsterdam/New York/Paris/ London/Milan/Tokyo/Buenos Aires, pp 4-6, 1969.
50. K.W.von Eickstedt and S.Rahman, Arzneim.Forsch., 19, 316 (1969).
51. K.Dziuba, Medizinsche Welt, 36, 1866 (1968).
52. V.M.Shpak and A.U.Scheglova, Pediatria, 47, 76 (1968).
53. N.A.Keuglov, Farmacol.Tokiskol., 31, 398 (1968).
54. D.U.Uaili, Zh.Nevropat.Psikhiat.Korsakov., 69, 589 (1969).
55. C.D.Neal, M.P.Collis and N.W.Imlah, Curr.Therap.Res., 11, 367 (1969).
56. J.L.Claghorn, Curr.Therap.Res., 11, 524 (1969).
57. H.Freeman and A.N.D.Frederick, Curr.Therap.Res., 11, 670

(1969).
58. P.Borenstein, C.Champion, Ph.Cujo, F.Gekiere, C.Olivenstein and P.Kramarz, Sem.Hopit.Paris, 45, 1301 (1969).
59. C.Laville and J.Margarit, Compt.Rend.Soc.Biol., 162, 869 (1968).
60. C.Laville and J.Margarit, Path.et.Biol., 17, 71 (1969).
61. G.Sierra, C.Acuna, J.Otero and G.Docampo, Int.J.Neuropharmacol., 8, 153 (1969).
62. A.Kalir, H.Edery, Z.Pelah, D.Balderman and G.Porath, J. Med.Chem., 12, 473 (1969).
63. A.A.Sugerman, J.Herrmann, M.O'Hara, G.M.Simpson, J.W.S. Angus and L.Deutsch, J.Clin.Pharmacol. J. New Drugs, 9, 183 (1969).
64. J.L.Claghorn and J.C.Schoolar, Curr.Therap.Res., 10, 279 (1968).
65. J.L.Benoit-Guyod, A.Boucherle, M.Benoit-Guyod, A.Dardas, R.Rupp, P.Eymard, G.Carraz, M.Boitard, S.Lebreton, H.Beriel and H.Meunier, Chim.Ther., 3, 336 (1968).
66. B.Loev, E.Macko and I.M.Fried, J.Med.Chem., 12, 854 (1969).
67. G.Pifferi, P.Consonni, S.Banfi and A.Diena, J.Med.Chem., 12, 261 (1969).
68. L.Bernardi, S.Coda, A.Bonsignori, L.Pegrassi and G.K.Suchowsky, Experientia, 25, 787 (1969).
69. G.K.Suchowsky and L.Pegrassi, Curr.Therap.Res., 11, 643 (1969).
70. S.Hayao, W.G.Strycker, B.M.Phillips, H.Fujimori and H.Vidrio, J.Med.Chem., 11, 1246 (1968).
71. C.P.Chien and R.M.Kaplan, Curr.Therap.Res., 11, 471 (1969).
72. R.Noyes, Dis.Nerv.Sys., 30, 318 (1969).
73. F.N.Pitts, Sci.American, 220, 69 (1969).
74. H.J.Grosz and B.B.Farmer, Arch.Gen.Psychiat., 21, 611(1969)
75. J.J.Schildkraut, New England J. of Medicine, 281, pp 197, 248, 302 (1969).
76. D.M.Loew and M.Taeschler, Int.Pharmacopsy, 1, 1 (1968).
77. R.C.Robichaud and K.L.Sledge, Life Sci., 8, 965 (1969).
78. D.D.Watt and P.R.Martin, Life Sci., 8, 1211 (1969).
79. Nature, 223, 999 (1969).

Chapter 2. Antidepressives and Stimulants

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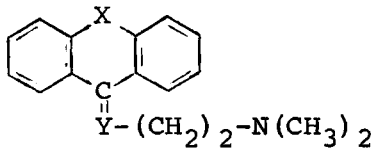
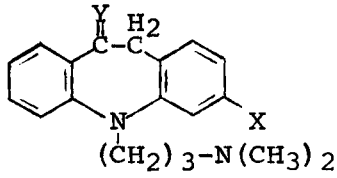
I. ANTIDEPRESSIVES

New Data and Structures - Considerable biological and clinical data have been reported on two structural modifications of amitriptyline (1). Doxepin (2, Sinequan^{R}) is a mixture of cis and trans isomers that exhibits a biological profile similar to that of amitriptyline, except that doxepin is more sedative, a property desirable in the treatment of agitated, depressed patients. The initial clinical comparisons have shown doxepin to compare favorably with amitriptyline and the standard amitriptyline-perphenazine combination.[1-3] Doxepin was placed on the U.S. market in 1969. Noxiptilin (3, Agedal^{R}) is one of the most active of a series of 51 oximinoethers. The chemical, biological (similar to amitriptyline) and clinical aspects of this drug were described in a series of papers.[4] Noxiptilin is an effective antidepressive agent now marketed in Germany.

In a clinical comparison, melitracen (4) was found to be less toxic and faster acting than imipramine.[5] Further clinical studies on two compounds related to imipramine (5) have been reported. In a double-blind study, chlorimipramine (6, Anafranil^{R}) and ketimipramine (7) produced a similar antidepressive effect.[6] The infusion of 6 produced an effect as early as the second day. The authors suggest a greater use of antidepressive drugs that can be administered intravenously.[7]





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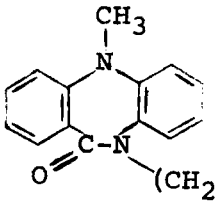
2	X=CH_2O	Y=CH	6	X=Cl	Y=H_2
3	X=CH_2CH_2	Y=N-O	7	X=H	Y=O
4	X=$C(CH_3)_2$	Y=CH			

A double-blind study comparing dibenzapin (8, Noveril^{R}) with imipramine showed no significant difference in effects.[8] A detailed pharmacological report on iprindole (9) has been published. Although it shared some of the biological properties of imipramine, iprindole was considerably less active in reversing reserpine-induced ptosis and hypothermia in mice and did not inhibit uptake of norepinephrine by rat heart or brain tissue.[9] In a clinical study, iprindole caused improvement in 75% of patients and caused fewer atropine-like side effects than did other antidepressives.[10] It failed to block REM sleep.[11]

In a preliminary evaluation, the indolinone UK-3540 (10), which has a profile of activity similar to that of the tricyclics but lacks antihistaminic and anticholinergic effects, exhibited antidepressive activity.[12] However, two side effects may seriously limit its use - scrotal discomfort and difficulty in initiating urination.[13] In a double-blind study, cyprolidol (11) was found to be less effective than imipramine in the treatment of endogenous depression.[14] In experimental animals, the substituted imidazoline DH-524 (12) showed some of the same effects as imipramine. Results of the initial clinical study of this compound were sufficiently favorable to merit a double-blind study.[15] The benzothiazepine Ro 5-8254 (13), which showed imipramine-like activity in pharmacological tests and catecholamine studies in animals, did not show antidepressive properties in a clinical trial.[16] Results of the initial human test of NC-0687 (14), a potent anticholinergic agent, were sufficiently promising to warrant further study. It is necessary to maintain the daily dosage of this drug below 30 mg to avoid side effects.[17] A preliminary report on the potent serotonin inhibitor and antihistaminic agent BC-105 (15), an analog of cyproheptadine, indicated that further studies of this drug in depressed patients were warranted.[18] In the first clinical experience with the benzimidazolinone AW 14 2446 (16), about 2/3 of the patients showed a positive antidepressive effect with remarkably few side effects.[19]

Butriptyline (17), a compound having structural features common to several psychotropic agents, was evaluated in 10 test procedures and showed a psychopharmacological profile similar to that of imipramine. The finding that butriptyline was more potent than imipramine in potentiating both ethyl alcohol- and hexobarbital-induced narcosis in mice may indicate that the compound possesses some sedative effects.[20] The



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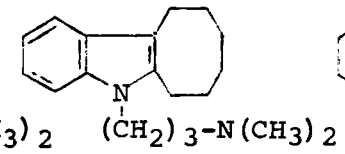

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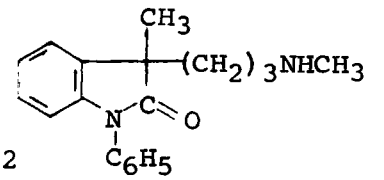

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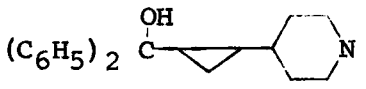

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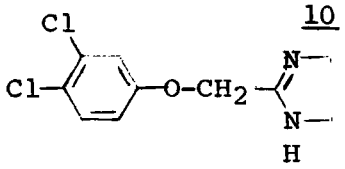

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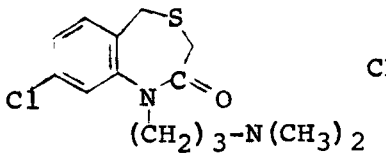

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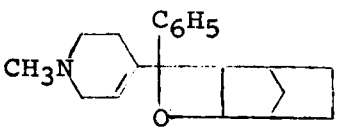

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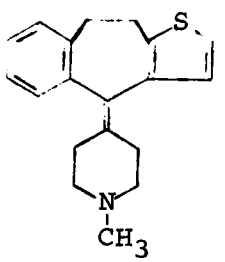

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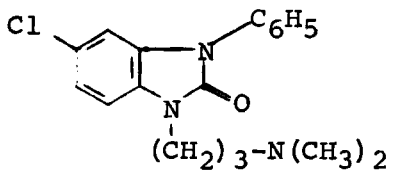

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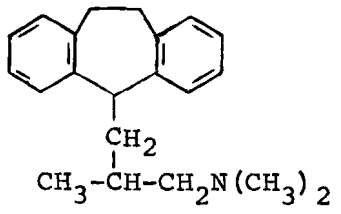
imidazoline Sch 12650 (18) prevents tetrabenazine-induced ptosis, reverses reserpine-induced hypothermia in mice and does not antagonize acetylcholine or norepinephrine.[21] A detailed pharmacological profile of the benzoxazinone FI 6654 (19) has been reported. The compound prevented the effects of reserpine in mice, rats, cats and monkeys, but did not antagonize tremorine- or oxotremorine-induced tremors in mice.[22,23] Studies on the peripheral pharmacology of fenazoxine (20) indicate that it inhibits the neuronal uptake of norepinephrine and tyramine.[24] Of a series of 16 substituted as-triazines, 21 was more active than imipramine in reversing reserpine-induced ptosis in mice. It also potentiated toxicity of amphetamine and prolonged hexobarbital sleeping time in mice.[25]

The biochemical and histochemical data for Lu 5-003 (22) and nine of its analogs show that these compounds have a selective action on the membrane pump mechanism of the central and peripheral catecholamine neurons and have little or no anticholinergic activity.[26] The synthesis and biological activity of 23, a "bridged" amitriptyline, and of several related structures have been reported. These tetracyclic structures did not show significant activity in reversing reserpine-induced hypothermia in mice.[27]



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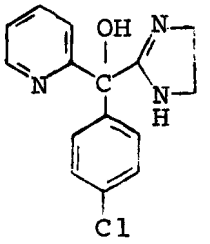

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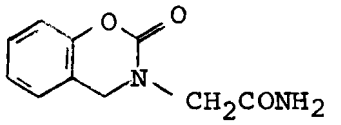

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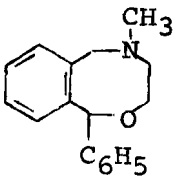

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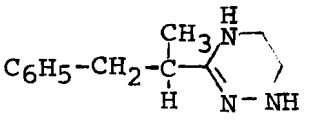

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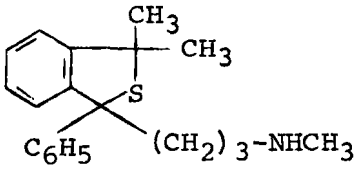

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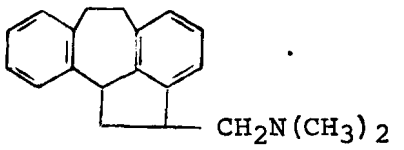


23

A controlled trial in unselected depressed patients showed that the combination of amitriptyline (25 mg) and perphenazine (2 mg) was more effective than imipramine (25 mg).[28] A double-blind study in acutely depressed patients, comparing a combination of amitriptyline (25 mg) and fluphenazine (0.5 mg) with amitriptyline (25 mg), showed that the combination was better in relieving the symptoms associated with depression.[29] Lithium carbonate was found to be highly effective in the treatment of mania but was less effective in the treatment of depression.[30] The lithium salts and the tricyclic antidepres-

sives have opposite effects on the monoamine-concentrating mechanisms of both rat brain and human platelets.[31] The numerous references to new inhibitors of monoamine oxidase has not been included in this presentation, since most biologists consider drugs that inhibit enzymes to be of limited usefulness in the treatment of depression. The recent discovery that MAO exists in at least four molecular forms in different areas of the rat and the human brains suggests the possibility of a selective inhibitor of MAO.[32,33]

Testing Procedures - The difficulties in devising a reliable test, or a series of tests, that can predict antidepressive activity become apparent from an examination of the extensive effort thus exerted. The ability of a compound to suppress the behavioral effects of yohimbine in the dog has been proposed as an indication of its potential clinical effectiveness as an antidepressive agent.[34,35] A study of 15 centrally acting drugs in four models of experimental depression in rodents showed that no drug or class of drugs is capable of blocking all four types of aggression at levels that are not neurotoxic.[36] Seven drugs were evaluated quantitatively for their antidepressive and tranquilizing activities using antagonism of reserpine-induced emesis and apomorphine-induced pecking in pigeons as test procedures. It is suggested that the results of these two tests form a simple and reliable combination for evaluating potential antidepressives.[37] A correlation is reported between the molecular features of tricyclic antidepressives and their inhibition of the uptake of norepinephrine by strips of rabbit aorta.[38] The effects of the tricyclics on the uptake and metabolism of intracisternally administered norepinephrine in the rat brain have been studied.[39] The degree of inhibition of uptake of serotonin by human platelets *in vitro* caused by 35 compounds related to imipramine was in general accord with clinical reports.[40] Results of a study of the effects of the tricyclic antidepressives on the depletion of intraneuronal stores of brain serotonin and catecholamines caused by α,4-dimethyl-*m*-tyramine in rodents suggests that blockage of serotonin re-uptake is involved in their mood elevating action whereas blockage of norepinephrine re-uptake is responsible for promoting drive in the depressed patients.[41] Studies in the rat indicate that the tricyclics cause a cholinergic response in the brain,[42] whereas data from a study in pigeons do not support a cholinergic mechanism.[43] In a study of 19 antihistaminic agents in rodents, no correlation was found between antihistaminic and antidepressive activities.[44]

Clinical Reviews - A series of three articles dealing with the classification of depression, neuropharmacological test procedures, and the limitations of present drugs used in the treatment of depression has been published.[45] The historical aspects of, and the biological basis for, the treatment of mood disorders is the subject of three reports.[46-48] Two other publications review the current status of the clinical use of psychotherapeutic drugs.[49,50] Extensive studies on the antidepressives and other pharmacological agents indicate that sleep and dream mechanisms are linked closely to serotonin and catecholamines.[51] Evidence supporting a simple clinical classification of anxiety and depressive states has been reported.[52] Also added to the literature is a tabulation of the interactions of the antidepressives with other drugs,[53] the observation of such side effects of antidepressives as iatrogenic epilepsy[54] and jaundice,[55] and of the increasing frequency of attempts of suicide by ingesting these agents.[56]

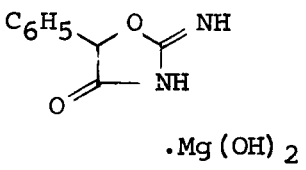
II. CENTRAL STIMULANTS

The greatest effort in this area involved the further study of magnesium pemoline(24, Cylert^{R}) and related structures. An extensive evaluation of the stimulant and analeptic properties of 24 showed it to be an active stimulant in mice, dogs, cats, and monkeys and an antagonist of the sedative effects of various tranquilizers.[57] All 19 analogs of pemoline in which the carbon atom to which the phenyl group is attached is part of a spirane system, were considerably less active than pemoline in increasing spontaneous activity in mice.[58] Mice receiving daily oral doses of 10 mg/kg of pemoline for 5 days showed an improvement in learning of the avoidance response, but did not show a clear-cut effect on retention of this response. Four analogs of pemoline were less effective in this study.[59] Self-aggressiveness induced by pemoline was observed in mice and rats about 8 hours after oral administration of the drug.[60] Three additional clinical studies on 24 were reported. Unhospitalized volunteers given 25-50 mg of 24, 100-200 mg of caffeine, or 15 mg of methylphenidate (25) all showed similar anti-fatigue effects.[61] Mentally handicapped workers given 50 mg of 24 daily did not increase their work output or the number of hours worked.[62] A group of inmates at a neuropsychiatric hospital receiving 10-20 mg of 24 daily showed a 17-18% improvement of memory.[63]

Phentermine (26), an analog of amphetamine, was found to

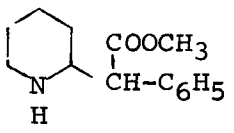
have the same spectrum of pharmacologic actions as dl-amphetamine. The latter was more active in reversing reserpine-induced ptosis in mice whereas both drugs caused a similar increase in blood pressure in the dog.[64] Oral administration of 26 or d-amphetamine to rats for 5 days effected marked elevations of blood pressure throughout the entire experiment.[65]

Methylphenidate and imipramine were more selective than atropine in antagonizing acetylcholine in four test procedures in the cat.[66] The partition coefficients and pK_a values for amphetamine and related drugs have been reported.[67] The absolute configuration of pipradol (27) was determined [68] and the effects of its optical isomers and of several other stimulants on endogenous catecholamines in mice has been studied.[69] The administration of 5 mg of pipradol to geriatric patients appeared to produce some beneficial effects.[70]



$\cdot Mg(OH)_2$

24



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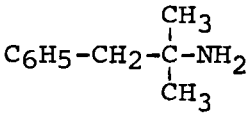

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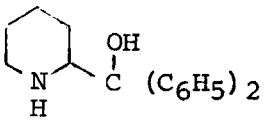

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SUMMARY

The wide variety of test procedures used in evaluating antidepressive agents makes it difficult to assess the potential of a candidate compound. There appears to be an adequate number of standardized test procedures to enable one to reasonably predict that a drug will produce an antidepressive effect in man similar to the slow-acting tricyclics; however, these test methods may not be sufficient in the search for the desired rapid-acting antidepressives.

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References

1. A symposium, Psychosomatics, 10, No.3 (Sec. II, p. 7 - Sec.I, p. 164), 1969.
2. B.J. Goldstein and D.F. Pinosky, Curr. Ther. Res., 11, 169 (1969).
3. G. Bauer and H. Nowak, Arzneimittel-Forschung, 19, 1642 (1969).
4. A symposium, ibid., 19, 833 (1969).
5. D. Biros, B.C. Schiele and D. Ferguson, Curr. Ther. Res., 11, 289 (1969).
6. W. Pöldinger, A. Gehring, A. Schäublin and R. Thilges, Arzneimittel-Forschung, 19, 492 (1969).
7. E. Pichler, R. Schigutt and H. Suchanek-Fröhlich, ibid., 19, 493 (1969).
8. J.M. Fielding, Med. J. Aust., 1, 614 (1969).
9. M.I. Gluckman and T. Baum, Psychopharmacologia, 15, 169 (1969).
10. D. Waxman, Brit. Med. J., 1, 254 (1969).
11. B.L. Baxter and M.I. Gluckman, Nature, 223, 750 (1969).
12. A. Canas-Rodriguez and P.R. Leeming, ibid., 223, 75 (1969).
13. D.M. Gallant, M.P. Bishop, R. Guerrero-Figueroa, L. O'Meallie and B. Moore, Curr. Ther. Res., 11, 296 (1969).
14. L.J. Hekimian, S. Gershon and A. Floyd, Arzneimittel-Forschung, 19, 955 (1969).
15. C.P. Chien and R.M. Kaplan, Curr. Ther. Res., 11, 471 (1969).
16. A.S. Leon, W.B. Abrams, M. Markowitz and E.C. Meisner, J. Clin. Pharmacol., 9, 399 (1969).
17. J.A. Yaryura-Tobias, L. White, A. Wolpert, L. Dana and S. Merlis, Curr. Ther. Res., 11, 574 (1969).
18. F.J. Ayd, Psychosomatics, 10, 51 (1969).
19. O.H. Arnold and H. Guss, Arzneimittel-Forschung, 19, 490 (1969).
20. K. Voith and F. Herr, Arch. Int. Pharmacodyn., 182, 318 (1969).
21. R. I. Taber, A. Barnett and F.E. Roth, Pharmacologist, 11, 247 (1969).
22. G.K. Suchowsky and L. Pegrassi, Arzneimittel-Forschung, 19, 643 (1969).
23. G.K. Suchowsky, L. Pegrassi and A. Bonsignori, Europ. J. Pharmacol., 6, 327 (1969).
24. J.R. Bassett, K.D. Cairncross, N.B. Hacket and M. Story, Brit. J. Pharmacol., 37, 69 (1969).

25. D.L. Trepanier, K.L. Shriver and J.N. Eble, J. Med. Chem., 12, 257 (1969).
26. A. Carlsson, K. Fuxe and B. Hamberger and T. Malmfors, Brit. J. Pharmacol., 36, 18 (1969).
27. E. Galantay, C. Hoffman and N. Paolella, J. Gogerty, L. Iorio, G. Leslie and J.H. Trapold, J. Med. Chem., 12, 444 (1969).
28. M.W. Browne, Brit. J. Psychiat., 115, 693 (1969).
29. J.F. Lowney and C.G. Cedarleaf, Curr. Ther. Res., 11, 271 (1969).
30. F.K. Goodwin, D.L. Murphy and W.E. Bunney, Arch. Gen. Psychiat., 21, 486 (1969).
31. D.L. Murphy, R.W. Colburn, J.M. Davis and W.E. Bunney, Life Sci., 8, 1187 (1969).
32. M.B.H. Youdim, G.G.S. Collins and M. Sandler, Nature, 223, 626 (1969).
33. G.G.S. Collins, M. Sandler, E.D. Williams and M.B.H. Youdim, ibid., 225, 817 (1970).
34. I. Sanghvi and S. Gershon, Life Sci., 8, 449 (1969).
35. I. Sanghvi, E. Bindler and S. Gershon, ibid., 8, 99 (1969).
36. R.D. Sofia, ibid., 8, 705 (1969).
37. F. P. Gupta, R.C. Saxena, O. Chandra and K.N. Dhawan, Psychopharmacologia, 15, 255 (1969).
38. R.A. Maxwell, P.D. Keenan, E. Chaplin, B. Roth and S. Batmanglidj Eckhardt, J. Pharmacol. Exp. Ther., 166, 320 (1969).
39. J.J. Schildkraut, G.A. Dodge and M.A. Logue, J. Psychiat. Res., 7, 29 (1969).
40. A. Todrick and A.C. Tait, J. Pharm. Pharmacol., 21, 751 (1969).
41. A. Carlsson, H. Corrodi and K. Fuxe and T. Hökfelt, Europ. J. Pharmacol., 5, 357 and 367 (1969).
42. J. Scheel-Krüger and A. Randrup, J. Pharm. Pharmacol,, 21, 403 (1969).
43. G.E. Vaillant, Amer. J. Psychiat., 125, 1600 (1969).
44. A. Barnett, R.I. Taber and F.E. Roth, Int. J. Neuropharmacol., 8, 73 (1969).
45. J.J. Schildkraut, New Eng. J. Med., 281, 197, 248 and 302 (1969).
46. B. Davies, Med. J. Aust., 1, 557 (1969).
47. F.M. Berger, Dis. Nerv. Syst., 30, 5 (1969).
48. W.G. Dewhurst, Proc. Roy. Soc. Med., 62, 1102 (1969).
49. L.E. Hollister, Clin. Pharmacol. Ther., 10, 170 (1969).
50. W. Weintraub, Mod. Treatm., 6, 793 (1969).

51. E. Hartmann, Biological Psychiatry, 1, 243 (1969).
52. D. Kelly and C.J.S. Walter, Brit. J. Psychiat., 115, 401 (1969).
53. D.A. Hussar, Amer. J. Pharm., 141, 123 (1969).
54. V. Dallos and K. Heathfield, Brit. Med. J., 1969, 4, 80.
55. D.H. Morgan, Brit. J. Psychiat., 115, 105 (1969).
56. J.W. Freeman, F. R. Mundy, R.R. Beattie and C. Ryan, Brit. Med. J., 1969, 2, 610.
57. N. Plotnikoff, F. Will and W. Ditzler, Arch. Int. Pharmacodyn., 181, 441 (1969).
58. M.R. Harnden and R.R. Rassmussen, J. Med. Chem., 12, 919 (1969).
59. C. Bianchi and E. Marazzi-Uberti, Psychopharmacologia, 15, 9 (1969).
60. E. Genovese, P.A. Napoli and N. Bolego-Zonta, Life Sci., 8, 513 (1969).
61. M.H. Orzack, C.L. Taylor and C. Kornetsky, Psychopharmacologia, 13, 413 (1968).
62. D.J. Greenblatt, A. DiMascio, M. Messier and B. Stotsky, Clin. Pharmacol. Ther., 10, 530 (1969).
63. J.A. Gomex, A.M. Bifet and R.A. Muray in "The Present Status of Psychotropic Drugs", A. Cerletti and F.J. Bove, Eds., Exerpta Medica Foundation, Amsterdam, Holland, 1969, pp. 421-424.
64. J. Yelnosky, R.E. Panasevich, A.R. Borrelli and R.B. Lawlor, Arch. Int. Pharmacodyn., 178, 62 (1969).
65. F. Kemper, Arzneimittel-Forschung, 19, 1702 (1969).
66. A.H. Tang and L.A. Schroeder, J. Pharm. Sci., 58, 257 (1969).
67. T.B. Vree, A.Th.J.M. Muskens and J.M. Rossum, J. Pharm. Pharmacol., 21, 774 (1969).
68. A. Shafi'ee and G. Hite, J. Med. Chem., 12, 266 (1969).
69. H.H. Wolf, D.E. Rollins and C.R. Rowland and T.G. Reigle, Int. J. Neuropharmacol., 8, 319 (1969).
70. I. Turek, A.A. Kurland, K.Y. Ota and T.E. Hanlon, J. Amer. Geriat. Soc., 17, 408 (1969).

Chapter 3. Hallucinogens

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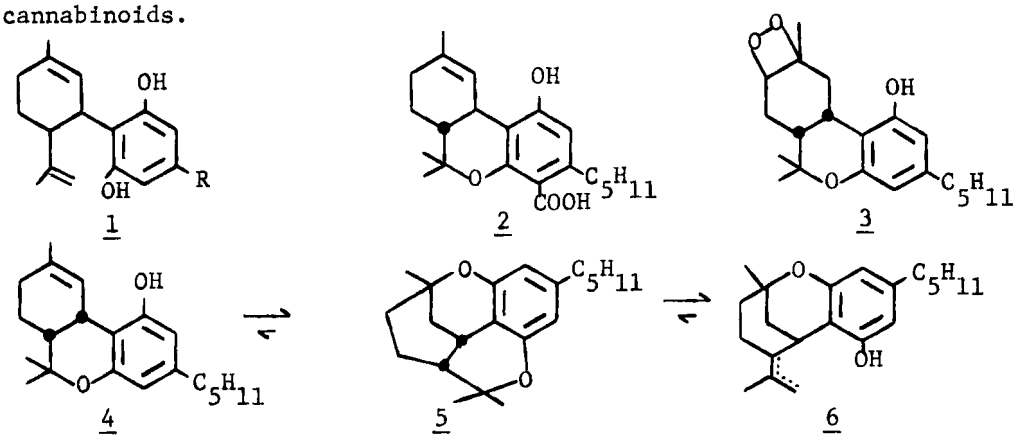
During 1969, the main progress in the field of hallucinogens has been in the chemistry of marihuana and ergot alkaloids, understanding of the biosynthesis of mescaline, evaluation of new psychotherapeutic agents, and in understanding their mechanism of action. Reviews have appeared on the available methods for studying the effects of hallucinogens,[1] the possible modes of action of psychotomimetics[2,3] and notably on hallucinogens of plant origin[4] (see Annual Reports, 1968, p. 19).

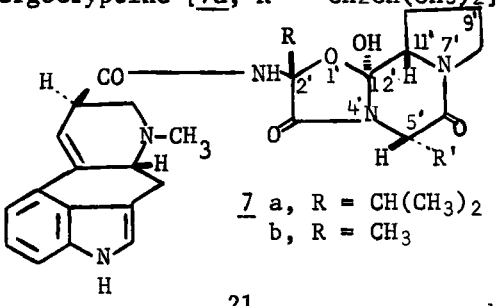
Chemistry - The understanding of the chemistry of cannabinoids has gathered momentum. A new component, cannabidivarin[5] (1, R = C_3H_7) was isolated from hashish. It is the first example of a natural cannabinoid with a n-propyl side chain in the aromatic ring. In addition to the usual Δ^1-tetrahydrocannabinolic acid, now referred to as "Δ^1-THC acid A", an isomeric, new tetrahydrocannabinolic acid[6] 2 ("Δ^1-THC acid B") has been isolated from some hashish samples. A general method utilizing methylmagnesium carbonate has been developed for the synthesis of cannabinoid acids and has been used for the synthesis of Δ^1-THC acid A, cannabidiolic and cannabigerolic acids.[7] A novel cannabinoid[8] (3) containing a cyclic peroxide has been isolated from the pyridine catalyzed reaction between citral and olivetol. It was also established that in the above reaction, other resorcinols can be substituted for olivetol to give various tetracyclic ethers of type (5) which lead to iso-THCs (6). Two mechanisms have been proposed for this reaction.[9] First example for the interconversion of Δ^1-3,4-*cis*-THC (4) to $\Delta^{1(6)}$-3,4-*trans*-THC (13) has been reported and it was further shown that 4, 5, and 6 are interconvertible.[10] On the basis of this equilibrium and other results, the authors have proposed an interpretation of acid-catalyzed transformations in cannabinoids.



Details for the synthesis of Δ^1- and $\Delta^{1(6)}$-THCs (13) from p-menthadienol have been published.[11] Tritiated Δ^1- and $\Delta^{1(6)}$-THCs and ^{14}C labelled $\Delta^{1(6)}$-THC were synthesized by two different methods.[12,13] Numerous detection methods for marihuana constituents,[14,15,16] including smoke condensate,[17]

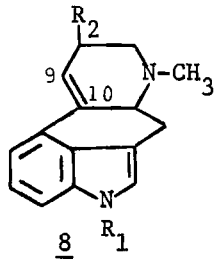
have been reported.

Alkaloids of ergotoxine group i.e. ergocristine (7a, R' = $CH_2C_6H_5$), α-ergocryptine [7a, R' = $CH_2CH(CH_3)_2$], β-ergocryptine [7a, R' = $CH(CH_3)\cdot CH_2CH_3$] and ergocornine [7a, R' = $CH(CH_3)_2$] were synthesized.[18] The absolute configurations at C-2' and C-12' in the peptide ring were identical to ergotamine (7b, R' = $CH_2C_6H_5$) alkaloids. Glycols, tweens[19] and surfactants and particularly dimethylsulfoxide[20] help in the production of ergot alkaloids by fermentation (*c. paspoli*) but are not involved in the biosynthetic process. In the latter, oxygenases[21] are involved. ^{14}C-labelled-D-lysergyl-L-alanine was incorporated in ergometrine but not in lysergic acid α-hydroxyethylamide.[22] Clavicipitic acid, a naturally occurring metabolite, was isolated from ergot cultures.[23] A review of biosynthetic mechanisms in ergoline alkaloid synthesis and their occurrence has appeared.[24] Ergolene derivatives continue to be prepared. Antiserotoninic activity[25,26] was found in 8 [R_1 = Me, R_2 = $CH_2NC_2H_5\cdot Bz$ and R_1 = H, R_2 = $CH_2CON(C_2H_5)_2$]. New compounds which have uterine contracting properties are lysergaldehydes (9,10-dihydro derivative of 8, R_1 = H or alkyl, R_2 = CHO) and their reaction products with phenylhydrazine, hydroxylamine, semicarbazide, etc.[27] A reagent for the epimerization of simpler amides of D-lysergic acid at C-8 was described.[28] A novel route for the synthesis of 2,3-dihydrolysergates from 5-bromoisatin was developed.[29]



7 a, R = $CH(CH_3)_2$
b, R = CH_3

Based on incorporation studies of labelled compounds, a biosynthetic route from tyrosine to mescaline was suggested.[30] These authors also proposed that the step, determining whether the common precursor 9 will be



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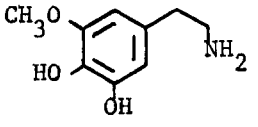

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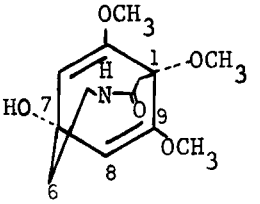
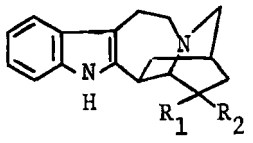


10

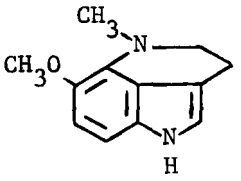
transformed into mescaline or tetrahydroisoquinoline alkaloids, is a methylation of 9 on the *meta*- or *para*-hydroxy group respectively. The former has been confirmed[31,32] but the latter is inconsistent with the findings of other workers.[33] Various Krebs cycle conjugates of mescaline were isolated and identified from peyote.[34] A novel compound 10 was isolated from the photolysis products of N-chloroacetyl mescaline. The nine-membered ring formed from C(1)-C(9) is in boat conformation[35] as shown by X-ray analysis.

A new synthetic approach, which was used for the synthesis of epi-ibogamine 11b, was extended to the synthesis of ibogamine (11a).[36] A different

total synthesis of 11b was also reported.[37] A new compound iboxygaine



11 a, R_1 = H, R_2 = C_2H_5
b, R_1 = C_2H_5, R_1 = H



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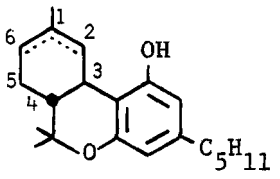


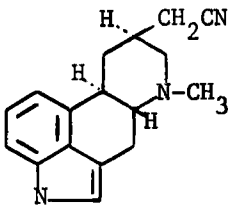
13 THC

hydroxyindolenimine[38] has been isolated from *Peschiera Lundii*. Two new β-carboline alkaloids[39] were found in some South American tryptamine containing hallucinogenic plants. This is interesting since β-carbolins, known MAO inhibitors, may thus be potentiating the hallucinogenic effects of tryptamines. Caffeine was isolated from *Banisteriopsis inebrians*[40] and a similar speculation is made that it might enhance hallucinogenic effects of harmine or harmaline. Psilocine was synthesized by the oxidation of N,N-dimethyltryptamine with Fenton-Cier reagent.[41] Psilocybin was produced by fermentation.[42] A new psychoactive indole 12 was synthesized.[43] In mice it shows only slight overt changes at 20 mg/kg s.c., but on the basis of tests in squirrel monkeys trained to discriminate between discs of different sizes, 12 was found to be twice as active as mescaline but much less active than the open chain analog N,N-dimethyl-5-methoxytryptamine. Stereoisomeric muscarines[44] were identified for the first time. A synthesis of Kavain[45] has appeared.

Biology - More reliable data on Δ^1- and $\Delta^{1(6)}$-THCs and other constituents of marihuana are forthcoming as synthetic material becomes available. In an anesthetized dog, both Δ^1- and $\Delta^{1(6)}$-THCs potentiate epinephrine and norepinephrine in all parameters.[46] The suggestion has been made that this may account for the euphoric effects in man. Neurochemical studies in the mouse[47] reinforce the suggestion that brain amines are involved in some way which is quite different from any other psychotropic drug. Administration of Δ^1-THC showed an increase of concentration of 5-HTP in whole brain, decrease of NE after low doses and an increase after high doses. The duration of effect on body temperature and spontaneous activity correlated generally with the changes in brain amines. Similarly LSD, psilocybine and JB-329 were shown to reduce the NE content of the rat hypothalamus at doses which produced the behavioral changes.[48] The cataleptoid reaction in rats and the behavioral changes in monkeys elicited by Δ^1- and $\Delta^{1(6)}$-THCs is reversed with (±) amphetamine[49] (0.5 mg/kg). Analgesic action of THCs in mice (see Annual Report 1968, p. 31) was not confirmed in terms of activity (only marginal) in the tail flick and hot plate tests. Fate and distribution studies with tritiated Δ^1-THC showed that in the rat it is eliminated very slowly and approximately 80% is excreted in metabolized form via feces and the remainder in urine.[50,51] Δ^1- and $\Delta^{1(6)}$-THCs, α-methyl and α,α-dimethyl derivatives of $\Delta^{1(6)}$-THC (13, C_5H_{11} replaced by $CH(CH_3)C_4H_9$ and $C(CH_3)_2C_4H_9$) showed the appearance of the spike and waves and flattening of the EEG of

rabbits in a neuropharmacological investigation.[52] On the other hand, cannabidiol (1, R = C_5H_{11}) showed only the flattening of EEG. It was suggested that this difference may be the EEG counterpart of the psychodysleptic action of THCs in man. Contrary to earlier belief, cannabidiol was found to cause severe motor deficit and ataxia when administered i.v. at 10 mg/kg in the rabbit.[52] Similarly cannabichromene[46] caused passiveness and loss of neuromuscular coordination in mice at 15-30 mg/kg (s.c.). A report[53a] has appeared which indicates that the teratogenicity of marihuana extract in animals is relatively low compared to other psychotomimetic agents. The effect of cannabis on chromosomes has been reported.[53b] Two independent reports[54,55] indicate that the mutagenic action of LSD has a threshold dose-response in *Drosophila melangaster*.[56] Injection of LSD was more efficient in inducing lethal mutations than its ingestion. From a study of the action of LSD on rabbits pretreated with DL-α-methyl-p-tyrosine,[57] it appears that the excitation of CNS and sympathomimetic actions of LSD are mediated by NE, whereas hypothermic action functions through a nonadrenergic mechanism. It was also demonstrated that the release of tritiated NE and 5-HT from electrically stimulated nerve endings in rat brain slices[58] was diminished. The suggestion was made that the mechanism of action of hallucinogens may be to inhibit the release of NE from the neuron, hence allowing its catabolism by MAO.[59] LSD was also shown to have an inhibitory effect on the spontaneous activity of neuronal units in the mid brain raphe nuclei of rats. This cannot be merely explained in terms of some non-specific stimulant action.[60] *In vitro* studies of LSD with purified calf thymus DNA[61] showed that it might cause changes in the conformation of DNA and thus make it more susceptible to enzymatic attack and breakage. Glycolysis was observed to be significantly increased in the rat brain, in a study of the effect of LSD on carbohydrate metabolism.[62]

A new class of antifertility agents was found in D-6-methyl-8-cyano-methylergolene[63] (14; "6605-VUFB"). Conception can be suppressed in rats by a single dose (10 mg/kg). The corresponding chloromethyl derivative (14, CH_2CN replaced by $-CH_2Cl$) was also active.[64] Ergotoxine and ergosine[65] showed similar activity.



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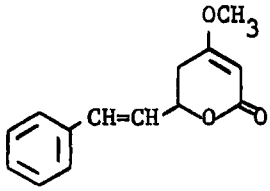
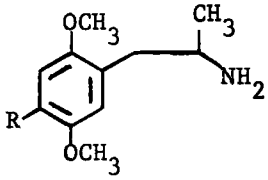


15 Kavain



16 a) R = CH_3 (DOM)
b) R = C_2H_5 (DOET)

Structure activity relationship in terms of spasmolytic activity of Kavain (15) and other active constituents of *P. methysticum*[66] was reported. They all inhibited or abolished contractions of isolated guinea pig ileum induced by acetylcholine, histamine, 5-HT or nicotine and were halt as active as papavarine sulfate. Reduction of the styryl double bond in Kavain reduced whereas unsaturation in the pyrone ring and substitution by methylenedioxy>

methoxy group in the benzene ring enhanced the spasmolytic activity.

In a study of the comparative effects of psychotomimetics on behavior in animals, the dog was considered to be the best species for use in psychopharmacological studies.[67] A method for the evaluation of hallucinogens, based on the performance of rats trained to swim through a Lashley-III underwater maze was described.[68] The response was found to be a function of dose and the rank order of potency of the compounds tested was similar to that in man. Similarly the effect of hallucinogens on the learned response of the squirrel monkey[69] was found to be debilitating and dose dependent. Another approach was to study the effect on a visual discrimination task in pigeons.[70] With LSD (300-750 μg/kg) and marihuana (20-30 mg/kg), the number of color errors were significantly increased. A novel method for the evaluation of hallucinogens and new drugs has been proposed.[71] It is based on a theory of association of EEG change and behavior as the scalp-recorded EEG in man provides a sensitive quantifiable index of central nervous changes associated with drug use. A method was developed which permitted monkeys to self administer drug solutions at will through i.v. catheters.[72] From a study of various CNS drugs, the authors concluded that psychological dependence appears to be the primary motive for drug abuse.

Reviews on anticholinergic hallucinogens[73] have appeared. The relation between clinical trials and the result of EEG techniques in animals was described.[74] Acetylcholine was found to complex molecularly with noradrenochrome.[75] This was suggested as a factor responsible for the hallucinogenic activity of the aminochromes.

Clinical - A comprehensive review of pharmacology of LSD in man has appeared.[76] A clinical comparison between LSD and Δ^1-THC was carried out in former opiate addicts.[77] The i.m. injection of LSD 0.5-1.5 μg/kg increased body temperature and blood pressure, lowered the threshold for knee jerk and dilated the pupils. In contrast, smoking of 75 and 325 μg/kg of Δ^1-THC produced none of these effects except a more marked tachycardia. Patients tolerant to LSD were not cross tolerant to THC, indicating the two drugs to be quite different. In an interesting paper, the problem of LSD and genetic damage is discussed.[78] The authors warn against facile interpretation of differences in the experimental data between a given small number of non-users and users of LSD. Moreover, their own data showed that not only LSD, but even more commonly used chemicals like aspirin and caffeine induced chromosome breakages in cultured Hela cells as well as human leukocytes. LSD and cannabis as possible teratogens in man[79] and the effect of marihuana on speech were discussed.[80] A study indicated that under the influence of marihuana as opposed to alcohol there is no impairment in simulated driving performance.[81] Since hallucinogens are generally associated with alterations in time and visual space perceptions, studies were reported on the effect of Psilocybin on visual perception, particularly the changes in brightness preference.[82] In another report changes in spatial distortion threshold were determined.[83]

The effect of the potent amphetamine analog DOM (STP; 16a) in 18 humans (2-14 mg) showed that the clinical syndrome resembled LSD, mescaline-type

drugs but produced unexpected sedation despite concomitant evidence of peripheral sympathetic stimulation. The plasma free fatty acids were increased with little effect on catecholamine excretion. Performance of psychometric tests was impaired. Chlorpromazine treatment attenuated the effects of DOM and the subjects rapidly developed tolerance to the drug.[84] In animal studies DOM was shown to affect the activation of the EEG response in the rabbit[85] and to accumulate in specific areas of the cat brain.[86] DOET (16b) (1.5 mg) was also tested in a double blind study in 10 subjects. It showed euphoria, and enhanced self-awareness in the absence of perceptual distortion or psychotomimetic changes. Its use as an adjunct to psychotherapy was suggested.[87]

An article on catnip has appeared.[88] Other articles of interest include the medicolegal considerations regarding hallucinogens,[89] correlations with reference to steroids and moods in schizophrenics and subjects treated with hallucinogens[90] and the clinical use of psychotherapeutic agents.[91]

REFERENCES

1. E. Jacobsen, Psychopharmacol. Dimensions Perspect. 175 (1968), edited by C.R.B. Joyce, Tavistock Publication, London, England.
2. A. Bebbington and R.W. Brimblecombe, Brit. Med. Bull. 25, 293 (1969).
3. V. Loppinet, Bull. Soc. Pharm. Nancy, No. 81, 18 (1969).
4. R.E. Schultes, Science 163, 245 (1969).
5. L. Vollner, D. Bieniek and F. Korte, Tetrahedron Lett. 145 (1969).
6. R. Mechoulam, Z. Ben-Zvi, B. Yagnitinsky and A. Shani, *ibid.*, 2339 (1969).
7. R. Mechoulam and Z. Ben-Zvi, J. Chem. Soc. D, 343 (1969).
8. R.K. Razdan and V.V. Kane, J. Am. Chem. Soc. 91, 5190 (1969).
9. V.V. Kane and R.K. Razdan, Tetrahedron Lett. 591 (1969).
10. R.K. Razdan and B. Zitko, *ibid.*, 4947 (1969).
11. T. Petrzilka, W. Haefliger, C. Sikemeir, Helv. Chim. Acta. 52, 1102 (1969).
12. C. G. Pitt and M. E. Wall, Tetrahedron Lett., 321 (1969).
13. J.L.G. Nilsson, I.M. Nilsson and S. Agurell, Acta Chem. Scand. 23, 2209 (1969).
14. R.F. Turk, H.I. Dharir and R.B. Forney, J. Forensic Sci. 14, 389 (1969).
15. L. Grlic, Bull. Narcotics 20, 25 (1968).
16. K.D. Parker, J.A. Wright, A.F. Halpern and C.H. Hine, *ibid.*, 20, 9 (1968).
17. H. Aramaki, N. Tomiyasu, H. Yoshimura and H. Tsukamoto, Eisei Kagaku 14, 262 (1968); Chem. Abstr. 70, 36122a (1969).
18. P.A. Stadler, St. Guttmann, H. Hauth, R.L. Huguenin, Ed. Sandrin, G. Wersin, H. Willems and A. Hofmann, Helv. Chim. Acta. 52, 1549 (1969).
19. A. Mizrahi and G. Miller, J. Bacteriol. 97, 1155 (1969).
20. W.J. Kelleher, B.K. Kim, A.E. Schwarting, Lloydia 32, 327 (1969).
21. E.O. Ogunlana, E. Ramstad and V.E. Tyler, Jr., J. Pharm. Sci. 58, 143 (1969).
22. G. Basmadjian, H.G. Floss, D. Groeger and D. Erge, J. Chem. Soc. D, 418 (1969).
23. J.E. Robbers and H.G. Floss, Tetrahedron Lett. 1857 (1969).
24. D. Groeger, Biosyn. Alkaloide, 486 (1969).
25. F. Troxler and A. Hofmann, Swiss 473,127; Chem. Abstr. 71, 91451c (1969).

26. F. Troxler, Fr. M. 4,946; Chem. Atstr. 71, 113139m (1969).
27. F. Troxler, J. Rutschmann and E. Schreier, Swiss 459,243; Chem. Abstr. 70, 58089x (1969).
28. A. Cerny and M. Semonsky, Collect. Czech. Chem. Commun. 34, 694 (1964).
29. M. Julia, F. LeGoffic, J. Igoler and M. Baillarge, Tetrahedron Lett. 1569 (1969).
30. J. Lundstrom and S.Agurell, *ibid.*, 3371 (1969).
31. A.G. Paul, K.L. Khanna, H. Rosenberg and M. Takido, J. Chem. Soc. D, 838 (1969).
32. G.J. Kapadia, Y.N. Vaishnav and M.B.E. Fayez, J. Pharm. Sci. 58, 1157 (1969).
33. A.R. Battersby, R. Binks and R. Huxtable, Tetrahedron Lett. 6111 (1968).
34. G.J. Kapadia and H.M. Fales, Chem. Commun. 1688 (1968).
35. O. Yonemitsu, N.H. Osamu, Y. Kanaoka, I.L. Karle and B. Witkop, J. Amer. Chem. Soc. 91, 4591 (1969).
36. M. Ikezaki, T. Wakamatsu and Y. Ban, Chem. Commun. 88 (1969).
37. P. Rosenmund, W.H. Haase and J. Bauer, Tetrahedron Lett. 4121 (1969).
38. B. Hwang, J.A. Weisbach, B. Douglas, R. Raffauf, M.P. Cava and K. Bessho, J. Org. Chem. 34, 412 (1969).
39. S. Agurell, Bo. Holmstedt, J.E. Lindgren and R.E. Schultes, Biochem. Pharmacol. 17, 2487 (1969).
40. F.D. O'Connell, Naturwissenschaften 56, 139 (1969).
41. M. Julia and F. Ricalens, C.R. Acad Sci. Ser. C, 269, 51 (1969) Chem. Abstr. 71, 70779a (1969).
42. A.Y. Leung and A.G. Paul, Lloydia 32, 66 (1969).
43. F.G.H. Lee, J.W. Daly and A.A. Manian, J. Med. Chem. 12, 321 (1969).
44. H.C. Eugster and E. Schleusener, Helv. Chim. Acta 52, 708 (1969).
45. Spezialchemie G.M.B.H. und Co., Fr. 1,526,691; Chem. Abstr. 71, 3275v (1969).
46. R.K. Razdan, V.V. Kane, H.G. Pars, J.L. Kucera, D.H. Reid and L.S. Harris, W.L. Dewey, J.F. Howes, Minutes 31st Meeting, Committee on Problems of Drug Dependence, NAS-NRC, 6135 (1969).
47. D. Holtzman, R.A. Lovell, J.H. Jaffe and D. Freedman, Science 163, 1464 (1969).
48. M.F. Sugrue, Brit. J. Pharmacol. 35, 243 (1969).
49. Y. Grunfeld and H. Edery, Psychopharmacologia 14, 200 (1969).
50. S. Agurell, I.M. Nilsson, A. Ohlsson and F. Sandberg, Biochem. Pharmacol. 18, 1195 (1969).
51. G. Joachimoglu, J. Kiburis and C. Miras, Prakt. Akad. Athenon 42, 161 (1967); Chem. Atstr. 70, 36345a (1969).
52. F. Lipparini, A. Scotti DeCarolis and V.G. Longo, Physiol. Behav. 4, 527 (1969).
53. (a) W.F. Geber and L.C. Schramm, Arch. Int. Pharmacodyn. Ther. 177, 244 (1969).
    (b) P.A. Martin, Lancet, 370 (1969); R.L. Neu, H.O. Powers, S. King and L.I. Gardner, *ibid.*, 675 (1969).
54. E. Vann, Nature 223, 95 (1969).
55. E.H. Markowitz, G.E. Brosseau Jr., E. Markowitz, Mutat. Res. 8, 337 (1969).
56. J.M. Tobin and J.M. Tobin, Dis. Nerv. Syst. 30, 47 (1969).
57. A. Horita and A.E. Hamilton, Science 164, 78 (1969).
58. I.J. Kopin, Pharmacol. Res. Commun. 1, 54 (1969).

59. B.E. Leonard and S.R. Tonge, Life Sci. 8, 815 (1969).
60. W.E. Foote, M.H. Sheard and G.K. Aghajanian, Nature 222, 567 (1969).
61. T.E. Wagner, *ibid.*, 222, 1170 (1969).
62. M. Torre and E. Vergani, Brain. Res. 12, 165 (1969).
63. K. Rezabek, M. Semonsky and N. Kucharczyk, Nature 221, 666 (1969).
64. M. Beran, J. Semonsky and K. Razabek, Collect. Czech. Chem. Commun. 34, 2819 (1969).
65. P.G. Mantle, J. Reprod. Fert. 18, 81 (1969).
66. R. Kretzchmar, H.J. Meyer, H.J. Teschendorf and B.Zoellner, Arch. Int. Pharmacodyn. Ther. 180, 475 (1969).
67. G.I. Milshtein, Zh. Evol. Biokhim. Fiziol. 4, 443 (1968); Chem. Abstr. 70, 18813x (1969).
68. E.T. Uyeno and C. Mitoma, Psychopharmacologia 16, 73 (1969).
69. E.T. Uyeno, Int. J. Neuropharmacol. 8, 245 (1969).
70. R.K. Siegel, Psychopharmacologia 15, 1 (1969).
71. M. Fink, Drugs Brain, 149 (1969). Edited by P. Black, Johns Hopkins Press, Baltimore, Md.
72. G. Deneau, T. Yanagita, M.H. Seevers, Psychopharmacologia 16, 30 (1969).
73. G.L. Abood, U.S. Public Health Serv. Publ. 1957-1967 (Pub. 1967), No. 1836, 683.
74. V.G. Longo, A. Scotti de Carolis, Progr. Brain Res. 28, 106 (1968).
75. L. Galzigna, C.R. Acad. Sci. Paris, Ser. D 268, 2498 (1969); Chem. Abstr. 71, 37260b (1969).
76. (a) D.X. Freedman, Ann. Rev. Med. 20, 409 (1969).
(b) L.E. Hollister, U.S. Public Health Serv. Publ. 1957-1967 (Pub. 1967) No. 1836, 1065.
77. H. Isbell and D.R. Jasinski, Psychopharmacologia 14, 115 (1969).
78. T. Kato and L.F. Jarvik, Dis. Nerv. Syst. 30, 42 (1969).
79. G. Carakushansky, R.L. Neu and L.I. Gardner, Lancet. 1, 150 (1969).
80. A.T. Weil and N.E. Zinberg, Nature 222, 434 (1969).
81. A. Crancer Jr., J.M. Dille, J.C. Delay, J.E. Wallace and M.D. Haykin, Science 164, 851 (1969).
82. R. Fischer, R.M. Hill and D. Warshay, Experientia 25, 166 (1969).
83. R.M. Hill, R. Fischer and D. Warshay, *ibid.*, 25, 171 (1969).
84. L.E. Hollister, M.F. Macnicol and H.K. Gillespie, Psychopharmacologia 14, 62 (1969).
85. V. Florio, F. Lipparini, A. Scotti de Carolis and V.G. Longo, Arch. Int. Pharmacodyn. Ther. 180, 81 (1969).
86. J.E. Idanpaan-Heikkila, W.M. McIsaac, T.B. Ho, G.E. Fritchie and W.L. Tansey, Science 164, 1085 (1969).
87. S.H. Snyder, L.A. Faillace and H. Weingartner, Arch. Gen. Psychiat. 21, 95 (1969).
88. B. Jackson and A. Reed, J. Amer. Med. Assn. 207, 1349 (1969).
89. S. Cohen, J. Clin. Pharmacol. 9, 5 (1969).
90. L.E. Hollister, *ibid.*, 9, 24 (1969).
91. L. E. Hollister, Clin. Pharmacol. Ther. 10, 170 (1969).

Chapter 4. Analgesic Agents

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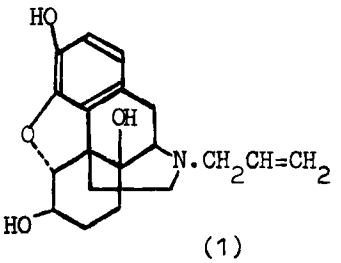
From the clinical standpoint the introduction of pentazocine as a non-addictive oral analgesic provided the major landmark in the year under review. Scientifically, the demonstration (see below) that optical resolution of active analgesics can separate analgesic activity and narcotic antagonism will probably rank as the year's major advance.

Apart from the review in this volume,[1] last year saw the publication of a book[2] on the medical basis of drug dependence as well as a comprehensive account[3] of codeine-level analgesics.

I Strong Analgesics A. Morphine-like compounds - In a masterly chemical exercise, Japanese workers[4] have shown that the isomer of morphine where the B/C rings are unnaturally trans fused possessed no analgesic activity but is more toxic than morphine; a finding which, by analogy with the related benzomorphans, was unexpected. Substituted benzoate esters[5] (phenolic OH) and the N-amino derivative[6] of morphine show little advantage over morphine itself. N-(1-Cyclohexenylmethyl)normorphine is claimed[6a] to be an antagonist-analgesic. Dihydronaloxone (1) shows[7] only one-tenth the activity of naloxone as an antagonist but has analgesic activity (writhing test) one-fifth that of morphine. Levallorphan has been found[8] to possess morphine-level activity in the clinic; its generally assumed usefulness in coprescription with meperidine has been proved false.[9]



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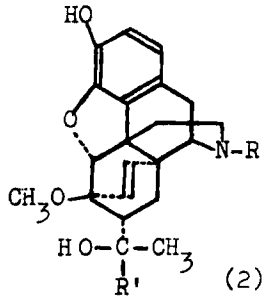

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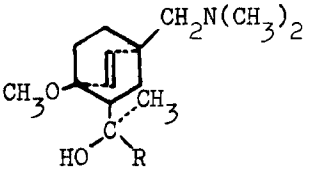

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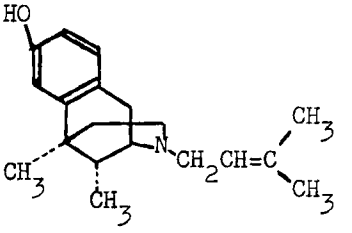


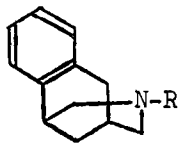
(4)

Experimental work continued on the potent thebaine analgesics but without the emergence of any clear clinical candidate. Etorphine (M99; 2, R=CH_3; R'=$CH_2CH_2CH_3$) induces tolerance in mice after a single dose[10] while its rapid absorption following oral administration in man[11] has been evidenced. The previously claimed[12] separation of analgesic and respiratory depressive effects in M218 (2, R=$CH_2CH=CH_2$; R'=$CH_2CH_2CH_3$) has been

refuted.[13] Much outstanding chemistry in the series has been reported[14] and details given[15] of the physical dependence liabilities of several of the compounds submitted to the National Institutes of Health. An attempt to isolate the essential analgesic component of these structures in the simple compounds (3) failed when they were found[14c] to be inactive.

B. Benzomorphans - Clinical trials of the d & l isomers of pentazocine (4) have shown[16] that both analgesic activity and side effects reside in the levo-isomer. Other trials, double-blind in many patients, confirm[17,18] the activity of the compound at about one-third that of morphine, while measurements of respiratory depression in treated patients suggest[19,20] this to be less than morphine at equianalgesic doses. The oral absorption has been determined[21] and metabolic breakdown found[22] to occur predominantly at the terminal methyl groups of the side chain. Reports of addiction to the injected form of pentazocine have been published[23,24,25] including one[26] to the oral form.

The many attempts to produce other potent benzomorphan-like structures are best exemplified by the comprehensive work[27] of Japanese chemists where the nine ring systems (5 - 9, n=1 and 2) were synthesised. The need for a nitrogen substituent in conventional benzomorphans (10) has been shown[28]



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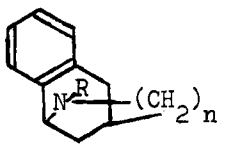

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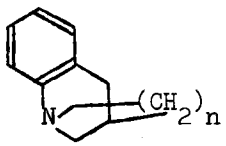

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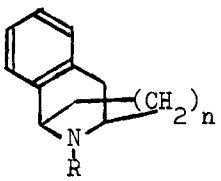

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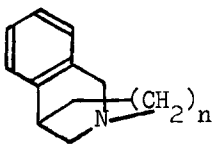

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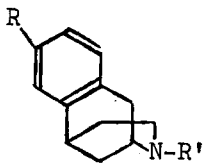
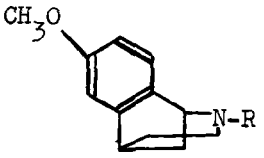


(10)

not to be critical: in (10, R=OH) activity is found when R' is either H or CH_3. This is one of the few cases where a secondary amine proved a good analgesic though slightly more toxic than the tertiary base. The latter (10, R=OH; R'=CH_3) though not possessing the usual 5 and 9 methyl groups showed supra-codeine level analgesia in mice and did not support morphine dependence in the Rhesus monkey. The presence of activity in the absence of these quaternary carbon atoms led the same school[29] to prepare the homologous B-norbenzomorphans (11, R=H and CH_3) which possessed codeine-level activity. The N-carboxamide (12) possessing one quaternary carbon, showed one-half the activity of morphine in the hot plate and Haffner tail pinch test but, surprisingly, was inactive in the rat tail flick; no addictive liability or repiratory depression was noted in monkeys.[30] In a

double blind clinical trial[30] it was superior to codeine. The introduction of ring methyl or phenyl groups into pentazocine-like structures



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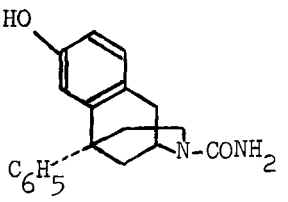

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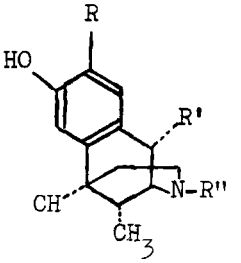
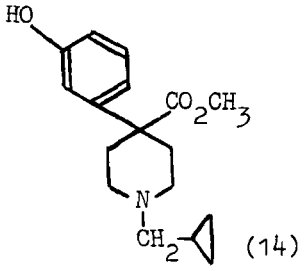


(13)

(13, R=CH_3; R'=H and R=H; R'=C_6H_5) gave[31] only weakly active compounds.

C. Meperidine-related Compounds - Much interest remains in compounds based on simple aza-alkane ring systems as potential analgetics. The bemidone analogue (14) has been shown[15] to have both analgesic and morphine-antagonist properties. The cis and trans forms of 3-methylmeperidine (15) were both active,[32] the cis ten times more potent than the trans. Several fentanyl analogues (16) showed good analgesia[33] but none with separation of analgesic and side effects.



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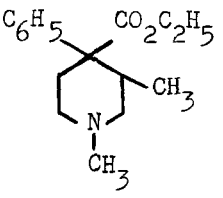

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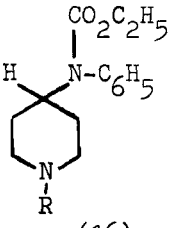

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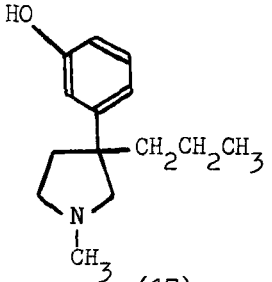

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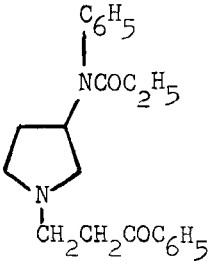

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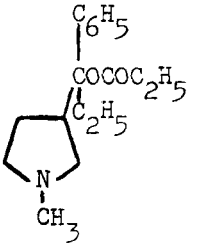
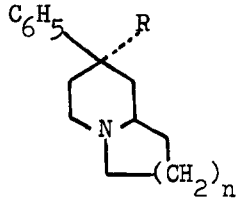


(19)

The pyrrolidine, profadol, (17) had one-quarter the activity of morphine in clinical trial.[34] Its capacity to precipitate the morphine abstinence syndrome was demonstrated[35] along with its failure to substitute for morphine in dependent subjects. Chronic parenteral administration to post-addicts, however, led to an abstinence syndrome of its own on withdrawal. A detailed account of the compound has appeared.[36] The amido-

pyrrolidine (18) was three times as active as morphine[37] whereas the ester (19) equated only with propoxyphene.[38]

The ring-fused piperidines (20, R=acyloxy and $CO_2C_2H_5$; n=1 and 2), prepared to evaluate the importance of steric factors and dealkylation mechanisms in meperidine-like compounds, proved to have less activity than the simple piperidines.[39]



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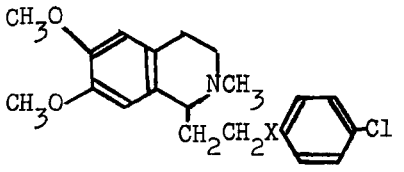

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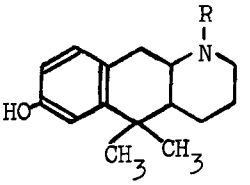
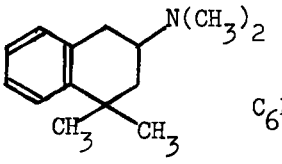


(22)

D. Miscellaneous - Several diverse structures, some quite unrelated to previously recognised analgetics, have been reported this year to possess activity. The methopholine analogues (21, X=O,S,SO and SO_2) are supra-codeine[40] but suppress morphine abstinence[15]; the benzquinolines (22, R=$CH_2CH=CH_2$ and cyclopropylmethyl) are claimed[41] to be analgesic narcotic



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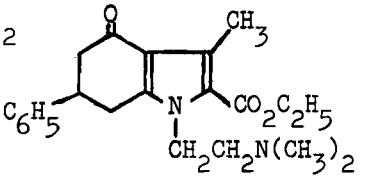

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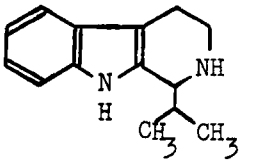

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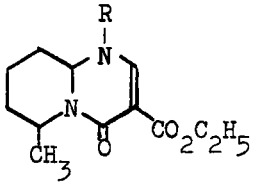

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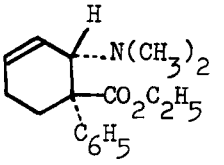

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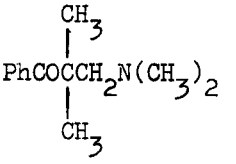


(28)

antagonists; the related aminotetralin (23) is supracodeine,[42] the indolone (24) morphine level[43] while the carboline, ICI 49,455, (25) resembles meperidine in level of activity but is poorly absorbed.[44] The 1,5-diazanaphthalenes (26, R=H and CH_3) were active orally in man at 0.3 - 1.5 g. daily and potentiated the action of morphine.[45] Two simple compounds, (27) and (28), have evidenced meperidine-level potency. The first of these (27, tilidine) demands a trans relationship between the basic and ester groupings for optimum activity, while reduction of the double bond renders it inactive.[46] The propiophenone (28), a rediscovered compound, showed one

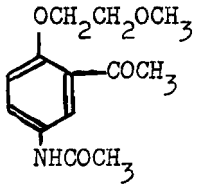
half the activity of morphine in the Haffner test.[47]

II Weak Analgesics - For complete coverage of this area Chapter 20 on anti-inflammatory drugs should be consulted.

A. Salicylates - Evidence has been given[48,49] that aspirin increases bleeding time and decreases blood platelet aggregation, probably by virtue of the acetoxy group. On the other hand, in oxidative phosphorylative uncoupling in mitochondria, the action is due to the salicylic acid moiety.[50]

B. Miscellaneous - The nephrotoxicity of phenacetin has been highlighted by a communication[51] instancing 14 patients with chronic renal disease after taking large quantities of the drug.

Novel compounds which have been reported include the aniline (29),[52] the azabicyclooctane (30),[53] the cycloheptimidazole (31)[54] and the phenylacetic acid (32),[55] all of which are claimed to show advantages over aspirin. The pyrrole (33) was supracodeine in rats but slightly less



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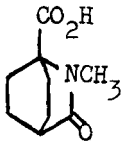

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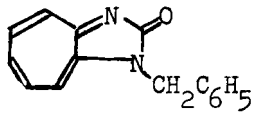

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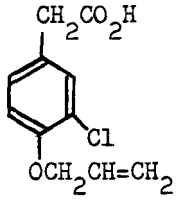

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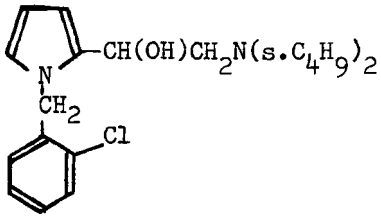

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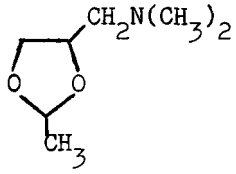
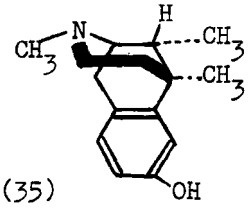


(34)

active than codeine in a clinical trial;[56] the simple dioxolane (34) proved one-half as active as codeine in rats.[57]

III Conformation of Strong Analgesics - An investigation of the apparently anomalous difference in conformation between the active stereoisomers of methadone and methadol has been made,[58] resulting in the design of an "ideal" receptor to accommodate both forms. The absolute configuration of metazocine (35) has been deduced[59] by the use of ORD techniques. Of



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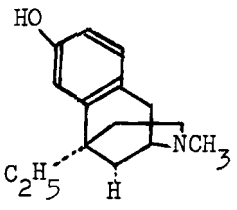

(36)

particular interest is the finding of May and his colleagues[60] that, in the benzomorphans (best example 36), potent analgesic activity along with antagonistic activity resides in the levo forms. Although the dextro forms are much weaker analgesics it is these that have the capacity to substitute for morphine in dependent animals. The physical dependence properties of the racemates of these compounds were low or absent, which suggested mutual antagonism between the constituent optical isomers.

IV Pharmacology - The complex nature of the pain response in mice and the measurement of its suppression by drugs is examined in an experimental comparison[61] of four standard methods widely used for the evaluation of analgetics. Support for the use of aconitine as a writhing agent,[62] and a further endorsement of acetic acid[63] for the same purpose, has been received. Using rigidly standardised mice, it has been possible to show[64] the diurnal variation in their susceptibility to morphine: maximum at midnight and minimum at 3 p.m. A valuable method by which the addictive liability of potential analgetics in mice can be assessed has appeared;[65] the withdrawal syndrome in these animals, obtained by surgically removing a previously implanted morphine pellet, is manifested by an uncontrollable jumping syndrome precipitated by morphine antagonists and suppressed by morphinomimetics (codeine was active at 25 mg/Kg s.c.).

The mechanism of pain conductance continues to excite attention. Both adrenergic and cholinergic mechanisms are postulated following the finding[66] of analgetic activity with oxytremorine, epinephrine and norepinephrine after intracerebral administration. No absolute correlation could be found between acetylcholine brain levels in mice following treatment with narcotics and narcotic antagonists or between such levels and rat tail flick data[67] although such a correlation was strongly indicated by the finding that inactive "analgetics" such as nalorphine (but not naloxone) showed good activity when given concomitantly with physostigmine.[68] The norepinephrine hypothesis was supported by the finding that cocaine (an adrenergic stimulator) was able to potentiate morphine analgesia while reserpine (a norephinephrine depletor) weakened it.[69] The administration of either iproniazid or tranylcypromine potentiates[70] the acute toxicity of strong analgesics.

V Clinical - Beecher[71] has given further support to the ischemic arm pain method for assessing new compounds in healthy volunteers. The same method has been used[72] to show that exposure to pain augments the development of tolerance to the analgetic effects of morphine in man. Both cutaneous electrical stimulation and cold pressor effects have been used[73] to distinguish aspirin from placebo.

REFERENCES

1. J.F. Cavalla in C.K. Cain, Ed., Ann. Rep. Med. Chem., 1968, p. 37, Academic Press, New York, N.Y., 1969.
2. Scientific Basis of Drug Dependence, H. Steinberg, Ed., J. & A. Churchill, London, 1969.
3. N.B. Eddy, H. Friebel, K-J Hahn and H. Halbach, Bull.Wld.Hlth.Org., 40,1(1969).

4. M. Takeda, H. Inoue and H. Kugita, Tetrahedron, 25,1839 and 1851(1969).
5. G. Selmeci, L.Szlavik, Z. Kaskoto, J.M. Lepenyene and A.I. Tothne, Khim.-Farm. Zh., 2,19(1968), Chem.Abstr., 70, 88011h(1969).
6. A. Modiri, J.G. Cannon and S.Y. Yeh. J.Med.Chem., 12,920(1969).
6a. French Pat., 550,538. Neth. Pat., 69,02547.
7. H.B. Dayton and H. Blumberg, Fed.Proc., 28,736(1969)
8. D. Piccinelli and B. Silvestrini, Brit.J.Pharmacol., 34,686P(1968).
9. J.C. Rouge, M.P. Banner and T.C. Smith, Clin. Pharmacol.Therap., 10,643 (1969).
10. M.W. Williams, C.S. Williams, L.A. Meeks and B.A. Gunning, Proc. Soc. Exptl.Biol.Med.,131,97(1969).
11. H.E. Dobbs, G.F. Blane and A.L.A. Boura, Europ.J.Pharmacol.,7,328(1969).
12. G.F. Blane, A.L.A. Boura, E.C. Leech, W.D. Gray and A.C. Ostenberg, J.Pharm.Pharmacol., 20,796(1968).
13. J.D. Bousfield and J.M.H. Rees, ibid., 21,631(1969).
14. (a) K.W. Bentley, D.G. Hardy, J.W. Lewis, M.J. Readhead and W.I. Rushworth, J.Chem.Soc.(C), 826(1969). (b) ibid., idem., pp.2225-2242. (c) K. Bentley, D.G. Hardy and P.A. Mayor, ibid., p.2385. (d) K.W. Bentley, J.D. Bower and J.W. Lewis, ibid., p.2569.
15. J.E. Villarreal and M.H. Seevers, Addendum to the Minutes of the 31st Meeting of the Committee of Problems of Drug Dependence, National Academy of Sciences, 1969.
16. W.H. Forrest Jr., E.G. Beer, J.W. Bellville, B.J. Ciliberti, E.V. Miller and R. Paddock, Clin. Pharmacol.Therap., 10,468(1969).
17. R. Paddock, E.G. Beer, J.W. Bellville, B.J. Ciliberti, W.H. Forrest and E.V. Miller, ibid., p.355.
18. M.E. Scott and R. Orr, Lancet,i,1865(1969).
19. S. Jennett, J.G. Barker, J.B. Forrest, Brit.J.Anaesth.,40,864(1968).
20. S. Lal, R.S. Savidge and G.P. Chhabra. Lancet,i,379(1969).
21. B.A. Berkowitz, J.H. Asling, S.M. Shnider and E.L. Way, Clin. Pharmacol. Therap., 10,320(1969).
22. B.A. Berkowitz and E.L. Way, ibid., p.681. (b) K.A. Pittman, D. Rose, R. Cherniak, A.J. Merola and W.D. Conway, Biochem. Pharmacol., 18,1673 (1969).
23. N. DeNosaquo, J.Am.Med. Assoc., 210,502(1969).
24. J. Neuschatz, ibid., 209,112(1969).
25. J.C. Schoolar, P. Idanpaan-Heikkila and A.S. Keats, Lancet, i,1263(1969)
26. R.H. Hart, ibid., ii,690 and 851(1969).
27. K. Mitsuhashi, S. Shiotani, R. Oh-Uchi and K. Shiraki, Chem.Pharm.Bull. (Tokyo),17,434(1969).
28. K. Kanematsu, M. Takeda, A.E. Jacobson and E.L. May, J.Med.Chem., 12, 405(1969).
29. A.E. Jacobson and M. Mokotoff, ibid., 13,7(1970).
30. F.B. Block and F.H. Clarke, ibid., 12,845(1969).
31. A. Ziering, N. Malatestinic, T. Williams and A. Brossi, ibid., 13,9 (1970).
32. A.F. Casy, L.G. Chatten and K.K. Khullar, J.Chem.Soc.(C), 2491(1969).
33. A.F. Casy, M.M.A. Hassan, A.B. Simmonds and D. Staniforth, J.Pharm. Pharmacol., 21,434(1969).
34. W.T. Beaver, S.L. Wallenstein, R.W. Houde and A. Royers, Clin.Pharmacol. Therap., 10,314(1969).

35. D.R. Jasinski, W.R. Martin and R. Hoeldtke, Fed.Proc.,28,735(1960).
36. R.E. Bowman, Chem. and Ind., 1077(1969).
37. G.C. Helsey, C.D. Lunsford, W.J. Welstead Jr.,R.F. Boswell Jr., W.H. Funderburk and D.N. Johnson, J.Med.Chem., 12,583(1969).
38. W.H. Funderburk, M.H. Fowell, D.N. Johnson and J.W. Ward, Arch.Intern. Pharmacodyn., 178,446(1969).
39. A.H. Beckett, R.G. Lingard and A.E.E. Theobald, J.Med.Chem.,12,563 and 569(1969).
40. T.H. Montzka, N.M.Cladel and J.D. Matiskella, ibid., p.575.
41. W.F. Michne and N.F. Albertson, ibid., p.402.
42. A.R. Martin, A.P. Parulkar, D.J. Gusseck, L.J. Anderson, G.L. Grunewald and A.I. White, J.Pharm., Sci. 58,340(1969).
43. K. Schoen, M. Finizio and I.J. Pachter, Abstracts 158th Meeting, Am. Chem.Soc., Div.Medicinal Chemistry, New York, N.Y. Sept.1969 No. 26.
44. (a) Ref. 2, p. 155. (b) D.S. Platts and B.L. Cockrill, Biochem. Pharmacol.,18,459(1969). (c) T.H. Flanagan, R.D. Broad, M.H. Rubinstein and A.R. Longworth, J.Pharm.Pharmacol., 21,129S(1969).
45. J. Knoll, Z. Meszaros and S. Furst, Abstracts IV Intern. Congress of Pharmacol., June 1969, p.455.
46. G. Satzinger, Annalen, 728,64(1969).
47. M.S. Atwal, L. Bauer, S.N. Dixit, J.E. Gearien, M.Megahy, R. Morris and C. Pokorny, J.Med.Chem.,12,994(1969).
48. T.H. Spaet, H. Al-Moudhiry and A.J. Marcus, Chem.Eng.News, April 28th, p.35(1969).
49. A.H. Youssef and P. Barkhan, Brit. Med.J., iii,394(1969).
50. L. Thompkins and K.H. Lee, J.Pharm.Sci., 58,102(1969).
51. D. Bell, D.N.S. Kerr, J. Swinney and W.K. Yeates, Brit.Med.J., iii, 378(1969).
52. H. Pelzer and E. Mueller, Arzneimittel-Forsch., 18,1400(1968).
53. Loevens, Brit. Patent 1,115,817.
54. S. Kobayashi and H. Takagi, Arzneimittel-Forsch., 18,939(1968).
55. N.P. Buu-Hoi, G. Lambelin, Cl. Gillet, J. Roba and M. Staquet, Naturwiss., 56,330(1969).
56. P. Procacci, G. Buzzelli, M. Grazzini & V. Monafo, Curr.Therap.Res., 11,647(1969).
57. D.A. McClure, Arch.Intern.Pharmacodyn., 179,154(1969).
58. P.S. Portoghese and D.A. Williams, J.Med.Chem., 12,839(1969).
59. A.F. Casy and A.P. Parulkar, ibid., 12,178(1969).
60. J.H. Ager, A.E. Jacobson and E.L. May, ibid., 12,288(1969).
61. C.P. Weller, I.Ibrahim and F.G. Sulman, Arch.Intern.Pharmacodyn., 176,176(1969).
62. T.N. Bhalla, J.N. Sinha, R.P. Kohli and K.P. Bhargava, Japan.J.Pharmacol.,19,179(1969).
63. R.I. Taber, D.D. Greenhouse, J.K. Kendell and S. Irwin, J.Pharmacol. Exptl.Therap., 169,29(1969).
64. R.W. Morris and E.F. Lutsch, J.Pharm.Sci., 58,374(1969).
65. E.L. Way, H.H. Loh and Fu-Hsiung Shen, J.Pharmacol.Exptl.Therap., 167,1(1969).
66. S.L. Handley and P.S.J. Spencer, Brit.J.Pharmacol., 35,362P(1969).
67. J.F. Howes, L.S. Harris, W.L. Dewey and C.A. Voyda, J.Pharmacol.Exptl. Therap., 169,23(1969).

68. L.S. Harris, W.L. Dewey, J.F. Howes, J.S. Kennedy and H. Pars, ibid., 169,17(1969).
69. (a) Yu.P.Vedernikov and I.I. Africanov, J.Pharm.Pharmacol., 21,845 (1969). (b) Yu.P.Vedernikov, Arch.Intern.Pharmacodyn., 182,59(1969).
70. K.J. Rogers and J.A. Thornton, Brit.J.Pharmacol., 36,470(1969).
71. G.M. Smith and H.K. Beecher, Clin.Pharmacol.Therap., 10,213(1969).
72. R.K. Ferguson and C.L. Mitchell, ibid., 10,372(1969).
73. B.B. Wolff, T.G. Kantor, M.E. Jarvik and E. Laska, ibid.,10,217(1969).

Chapter 5. Anorexigenic Agents

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Introduction - No major advances were reported in the field of anorexigenic agents during the two year period 1968-1969. Obesity and weight control continues to be a problem of great concern. An international symposium on amphetamines has been held[1] and a general review on obesity and its control has been published.[2] It has been estimated that at least 30% of the present adult population of the United States may be considered obese.[3]

Existing anorectic drugs are far from being ideal. However, their therapeutic use in the overall management of obesity continues to be recommended.[4] Certain non-sympathomimetic drugs such as digitalis, cardiac glycosides, thyroid hormone and diuretics are not recommended in the treatment of simple obesity.[5] Formulations of digitalis and thyroid extracts have been heavily criticized[6] in the published literature and are now banned by the Food and Drug Administration.[7]

Pharmacology and Biochemistry - Electrophysiologic studies in cats[8] and rabbits[9] suggest that the appetite-inhibiting action of fenfluramine (Ia) like that of d-amphetamine, is due to the stimulation of the ventromedial nucleus of the hypothalamus. However, unlike d-amphetamine, fenfluramine also depresses the cortex but does not produce global hyperactivity.[8]

A comparative study of d-amphetamine, chlorphentermine (II) and fenfluramine in aurothioglucose obese mice suggests that these compounds elicit their anorectic activity via the lateral hypothalamic center.[10] This study supports earlier reports that amphetamine increases the eating threshold elicited by stimulation of the lateral hypothalamus.[11] However, crystalline amphetamine implanted into the lateral hypothalamus did not affect food intake.[12] Bilateral and unilateral injections of solutions of amphetamine into the lateral hypothalamic area of rats depress their food intake.[13]

Recent studies, attempting to elicit the mechanism of actions of anorexigenics, involve the role of fats. Controversial views exist in regard to free fatty acid (FFA) mobilization by various anorexigenic agents. A rise in plasma FFA is reported for amphetamine,[14] phenmetrazine (III),[15] and fenfluramine.[16] Other investigators,[17] however, comparing d-amphetamine, phenmetrazine and RO4-5282 (IVb) have found that large doses depress the appetite but do not have significant effects on plasma FFA levels, in their obese patients.

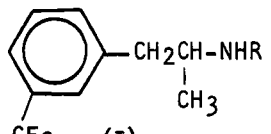
Clinical studies[18] utilizing 1-^{14}C labeled palmitic acid complexed to human albumin, gave no evidence of lipid mobilization in the obese subjects at rest or during work.

The relationship of corticosteroids to obesity has been investigated. Some obese patients have been known to have elevated corticosteroid levels.[19] A significant increase in plasma corticosteroid levels has been found in healthy young males after ingestion of d-amphetamine or intravenous administration of methamphetamine (IVa).[20] The latter compound was also associated with an increase in immunoreactive corticotropin level. It was suggested that these effects may represent a direct action of these drugs on hypothalamic or mid-brain receptors.

Serum insulin studies[21] have led to the conclusion that an abnormal insulin secretion or utilization may precede obesity. In another study,[22] the high blood levels of insulin-like activity found in obese subjects are reduced to normal limits when the subjects approached their ideal weight.

The effect of a β-blocking agent, propranolol, on some activities of amphetamine in mice has been studied;[23] more support is given to the hypothesis that amphetamine anorexia is independent of other effects induced by the drug.

Phenalkylamine Derivatives - The bulk of the published work on anorexigenic agents continues to be directed to derivatives of amphetamine. Fenfluramine (Ia) continues to evoke considerable interest. Pharmacologic studies on the depletion of cardiac[24] and brain[25,26] norepinephrine stores as well as other catecholamines have been reported. A stimulant component of fenfluramine and a new benzyloxy derivative S-992 (Ib) has been demonstrated[27] under conditions providing an increased availability of catecholamines produced by dopa in monoamine oxidase inhibitor pretreated mice. It (Ia) has been found to be at least 8 times as potent as d-amphetamine on acetylcholine induced muscle contractions[28] suggesting that it does not act on adrenoceptive receptors and thus must be exerting its action through a different part of the muscle-cell membrane or within the wall itself. In a limited clinical study, fenfluramine was found to increase forearm blood flow and muscle glucose uptake. It was concluded that the drug mimics the metabolic pattern of lean subjects who have greater muscle glucose uptake than fat persons.[29]

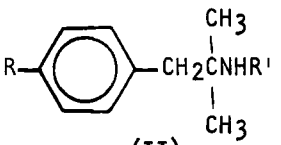


(I)

R=

a C_2H_5

b $CH_2CH_2OCH_2C_6H_5$

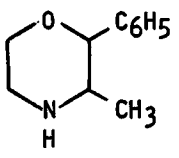


(II)

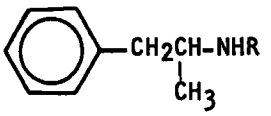
	R=	R'=
a	Cl	H
b	Cl	$COSC_2H_5$
c	F	CH_3
d	Cl	CH_2COCH_3

Clinically (Ia) has been claimed to be the most suitable anorectic drug for patients who need to lose weight.[29,30] However, it is contraindicated for depressed obese patients.[29] The absolute configuration of (+)-fenfluramine has been established as being identical to that of (+)-amphetamine.[31] The major metabolites of fenfluramine in man are m-trifluoromethylhippuric acid, (66-93%), demethylated fenfluramine and the unchanged drug.[32]

The distribution and metabolic fate of ^{14}-C labeled chlorphentermine (IIa) has been studied in mice and rats.[33] The drug is not stored in the adipose tissue; most of the drug is excreted in the urine in 24 hours. Rats excreted 70-90% as the unchanged drug, presumably due to blockage of both p-hydroxylation and deamination of the metabolic pathways. However, female mice excreted only about 25% of unchanged drug and 60% of an unidentified acidic conjugate. Clinical studies with chlorphentermine have been reported in teen-agers,[34] young adults[35] and postpartal patients.[36] In young adults chlorphentermine significantly decreased "critical flicker frequency" suggesting a depressant effect on the CNS.[37] Several new derivatives of chlorphentermine have been reported to produce anorexia. A thiocarbamate derivative (IIb) related to cloferex has been found to be 20 times less toxic but 2 to 3 times less active than chlorphentermine.[38] The p-fluoro derivative (IIc) produces anorexia but only at doses producing CNS stimulation.[39] A keto derivative (IId) of chlorphentermine is reported to be a useful anorexient free of undesirable side-effects.[40]



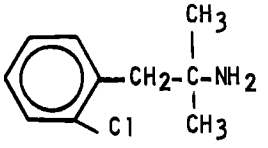
(III)



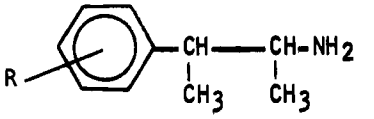
(IV)

	R=
a	CH_3
b	$(CH_2)_3Cl$
c	$(CH_2)_2CN$

Voranil, SU-10568 (V) an ortho chlorosubstituted analogue was reported to be as effective an anorexigen as its structural analogue chlorphentermine.[41] A clinical trial of (V) at daily doses of 50 mg, revealed effects similar to those obtained with chlorphentermine.[42]



(V)



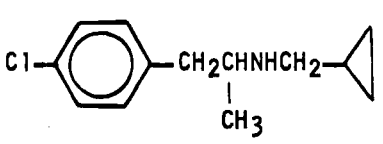
(VI)

	R=
a	m-Cl
b	p-Br

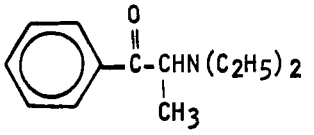
Of a series of 35 α- and α,β-alkyl substituted phenethylamines being investigated for CNS effects, two compounds, B-1268 (VIa) and B-1279 (VIb) were reported to be comparable to chlorphentermine in reducing food and bouillon consumption in rats.[43] dl-p-Chloro-N-(cyclopropylmethyl)-α-methylphenethylamine A-31960 (VII) has been compared to methamphetamine and diethylpropion (VIII) in man. It was found to have comparable activity in respect to appetite suppression and CNS stimulation[44] to methamphetamine and diethylpropion.

The pharmacology of RO4-5282 (IVb) has been published. In the cat, its appetite suppression has been shown to be due to a direct effect on the ventrolateral nucleus of the hypothalamus.[45]

Human metabolism of 1-^{14}C-diethylpropion has been described.[46] Twenty-one metabolites have been identified. The major metabolite, hippuric acid, represented about 27% of the radioactivity excreted in the urine between 8 and 12 hours after ingestion of the drug.

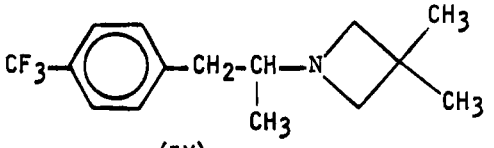


(VII)

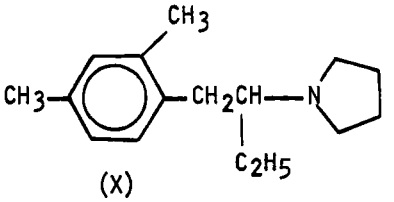


(VIII)

An N-cyanoethyl derivative (IVc) of amphetamine has been examined clinically and reported to be effective in reducing appetite. It has no cardiovascular effects, caused little central stimulation and did not cause insomnia.[47] A series of azetidine derivatives has been described, and one of these (IX) was reported to have about one-third the activity of methamphetamine.[48]



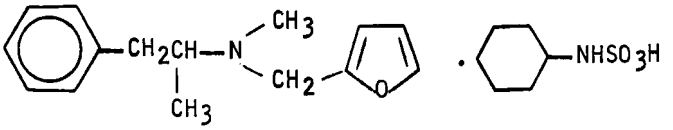
(IX)



(X)

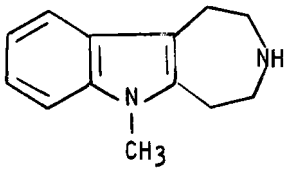
1-(2,4-Dimethylphenyl)-2-pyrrolidinobutane (X) is reported to resemble phenmetrazine in its profile in rats and mice but is relatively less toxic.[49]

A study of the metabolism of furfenorex (XI), in the rat, discloses that about 10-20% of the drug could be accounted. The principal urinary metabolites identified by VPC were amphetamine and N-methyl amphetamine as well as lesser amounts of their para-hydroxylated derivatives.[50]

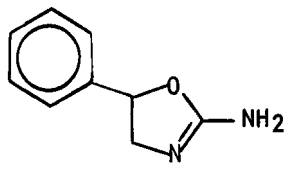


(XI)

A new indole derivative, U-22394 (XII) has shown anorexigenic activity in mice comparable to that of d-amphetamine. Other exhibited activity includes, hypothermia, blocking conditioned avoidance reflex, antagonizing aggressive behavior and displaying tryptamine-like activity, in mice.[51] No antipsychotic activity was noted in a clinical study with psychotics.[52] However, the patients did lose weight, suggesting a possible anorexigenic effect.



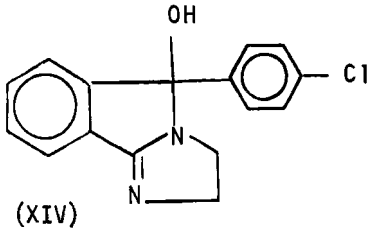
(XII)



(XIII)

Conflicting evidence has been reported on the roles of anorexigens in pulmonary hypertension.[53,54] It has been suggested that aminorex (XIII) may be implicated in the development of pulmonary hypertension in dogs[55,56] and in humans.[57-61] On the other hand, 4,000 patients receiving aminorex showed no such side effect.[62] In a series of studies that included treatment of obese patients with chronic chest diseases, with fenfluramine, no evidence of pulmonary hypertension was found.[54] Therefore, to date no definite correlation can be made between appetite suppressants and the development of pulmonary hypertension.

Non-Phenethylamine Derivatives - The search continues for anorectic compounds not structurally related to phenethylamines. An isoindole derivative, 5-hydroxy-5-p-chlorophenyl-2,3-dihydro-5H-imidazo[2,1-a]-isoindole, 42-548 (XIV) has been reported to have moderate anorexic effects in monkeys at doses below those producing significant CNS stimulation.[63] In obese male patients 42-548 was found to be an effective anorexigenic agent with no significant effects on sleep or mood.[64]



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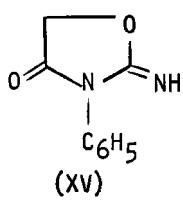
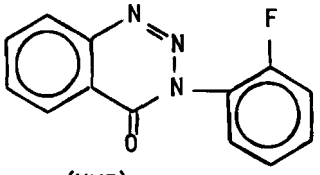


(XV)

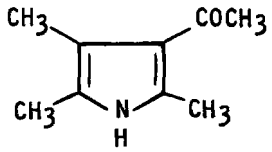
Pondex (XV) is an oxazolidine derivative, reportedly being used clinically in Hungary, found to be comparable to phenmetrazine in reducing weight of obese women on a restricted diet.[65]

3-(2'-Fluorophenyl)-3H-benzo-1,2,3-triazin-4-one (XVI) is reported to be twice as effective as chlorphentermine in suppressing appetite in rats. No toxic effects were noted.[66]

A polysubstituted pyrrole (XVII) has been reported to have weak anorexigenic activity in dogs.[67]



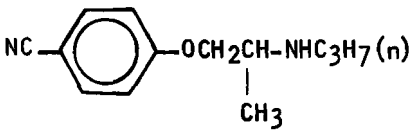
(XVI)



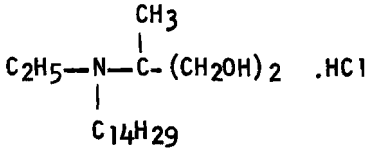
(XVII)

1-(4'-Cyanophenoxy)-2-n-propylaminopropane (XVIII) has been compared to phendimetrazine in dogs and found to effectively reduce food consumption at one-seventh the dose that produces CNS effects.[68]

2-(N-Tetradecyl-N-ethylamino)-2-methyl-1,3-propane-diol hydrochloride (XIX) significantly inhibited the growth rate of rats. Activity was due to the inhibition of intestinal absorption as well as to irritating effects on the esophagus.[69]



(XVIII)



(XIX)

Miscellaneous - Several reports have appeared on a substance which may be isolated from the urine of rats, man and other animals. This Fat-Mobilizing Substance (FMS) (FMS 1A) has been compared with adrenaline and amphetamine in rats. The urinary anorexigen, FMS 1A, had a prolonged anorexigenic effect which was still evident after 48 hours.[70] Properties of this substance continue to be investigated.[71] In a limited clinical trial, evidence was obtained that FMS injected every other day produced significant weight loss, without toxic effects.[72]

Certain bile acids, deoxycholic and chenodeoxycholic acid, have been shown to decrease the desire for food in grossly obese patients.[73] The proponents for the use of human chorionic gonadotrophin as a useful adjunct in the treatment of obesity[74,75] have been criticized.[76]

Clinical studies have been reported on the effects of hypoglycemic biguanides on weight reduction.[77-79] Recent studies suggest that tolerance develops within 16-32 weeks after Metformin therapy.[77]

Studies on the pharmacokinetics of known anorexigens have been reported[80] in an attempt to further understand their activity, from a physical scientist's point of view. Physiologists and pharmacologists are contributing to this general subject by developing simpler and more rapid methods for the screening of these agents.[81-82]

References

1. Proceedings of an International Symposium on Amphetamines and Related Compounds. Milan, Italy (1969) [In Press]
2. P. Vinay, P. Pesant, H.F. Beauregard, J. Bervier and C. Dufault, L. Union Med. Canada 98, 781-796 (1969).
3. S.H. Waxler and M.F. Leef, Geriatrics, 24, 98-106 (1969).
4. R.I.H. Wang and R. Sandoval, Wisconsin Med. Journal, 68, 219 (1969).
5. Anonymous, J.A.M.A., 204, 328 (1968).
6. A.A. Kattus, Jr., B.W. Biscoe, A.M. Dashe and J.H. Davis, Arch. Intern. Med., 122, 298-304 (1968).
7. J.L. Goddard, Fed. Regist. 33, 5616 (1968).
8. M.H. Foxwell, W.H. Funderbunk and J.W. Ward, J. Pharmacol. Exp. Therap., 165, 60-70 (1969).
9. H. Schmitt and J.C. LeDouarec, Therapie, 20, 111-120 (1965).
10. A.H. Abdallah, Arch. Int. Pharmacodyn., 176, 395-402 (1968).
11. W.F. Thode and H.J. Carlisle, J. Comp. Physiol. Psychol., 66 (2), 547-8 (1968).
12. P. Stark and C.W. Totty, J. Pharmacol. Exp. Therap., 158, 272 (1967).
13. D.A. Booth, Nature, 217, 869-70 (1968).
14. E.J. Pinter and C.J. Pattee, J. Clin. Inves., 47, 394-99 (1968).
15. L. Laurian, Z. Oberman and M. Herzberg, Israel J. Med. Sci., 4, 311-14 (1968).
16. Z.N. Gaut, R. Pocelinko, W.B. Abrams and C. Dalton, J. Clin. Pharm., 9, 315-20 (1969).
17. G.L.S. Pawan, Lancet, 1, 498-500 (1969).
18. P.Bjorntorp, H. Bergman and E. Varnauskas, Acta Med. Scand., 185, 351-56 (1969).
19. G. Hollifield, Am. J. Clin. Nutr., 21, 1471-74 (1968).
20. G.M. Besser, P.W.P. Butler, J. Landon, and L. Rees, Brit. Med. Jour., 4, 528-30 (1969).
21. B.R. Boshell, H.B. Chandalia, R.A. Kreisberg and R.F. Roddam, Am. J. Clin. Nutr., 21, 1419-28 (1968).
22. S. Szanto, Proc. Nutr. Soc., 27, 11A-12A (1968).
23. P. Mantegazza, K.M. Naimzada, and M. Riva, Europ. J. Pharmacol., 4, 25-30 (1968).
24. G. Sipes, H.H. Smookler and J.P. Buckley, Pharmacologist, 9, 198 (1967).

25. R. Ziance and W.J. Kinnard, Pharmacologist, 9, 244 (1967).
26. R. Ziance and J.P. Buckley, Pharmacologist, 11, 264 (1969).
27. S. Jespersen, A. Bonaccorsi and S. Garattini, J. Pharm. Pharmacol., 21, 558-59 (1969).
28. I.M. Coupar, A. Hedges, H.L. Metcalf and P. Turner, Lancet, 1, 925-26 (1969).
29. R. Gaind, Brit. J. Psychiat., 115, 963-64 (1969).
30. L.J.P. Duncan and J.F. Munro, Practitioner, 200, 167-73 (1968).
31. A.H. Beckett and G.L. Brookes, Tetrahedron, 24, 1283-87 (1968).
32. R.B. Bruce and W.R. Maynard, Jr., J. Pharm. Sci., 57, 1173-76 (1968).
33. B. Dubnick, C.A. Towne, J.M. Hartigan and G.E. Phillips., Biochem. Pharmacol., 17, 1243-50 (1968).
34. J.L. Rauh and R. Lipp, Clin. Pediat., 7, 138-40 (1968).
35. P.C. Trickett and J.H. Weir, Pac. Med. Surg., 76, 35-9 (1968).
36. S. Duckman, W. Chen and J.H. Weir, Curr. Ther. Res., 10, 619-25 (1968).
37. H. Misiak and J.M. Smith, Curr. Ther. Res., 10, 641-48 (1968).
38. K.H. Boltze, R. Kopf and D. Lorenz, German Patent 1,268,133 (1968).
39. E.D. Bergmann and Z. Goldschmidt, J. Med. Chem., 11, 1242-44 (1968).
40. A.L. Langis, U.S. Patent 3,456,011 (1969).
41. D.V. Finocchio, U.S. Patent 3,415,937 (1968).
42. J.J. Rodos, J. Amer. Osteopath. Ass., 69, 161-64 (1969).
43. K. Binovic, S. Vrancea, D. Grandet, J.M. Lebourgh and R. Porguet, Chim. Therap., 3, 313-20 (1968).
44. R.L. Herting, R.J. Powers, and G. Dillion, Pharmacologist, 11, 264 (1969).
45. J.E. Blum, Arzneimittel-Forsch., 19, 748-55 (1969).
46. E.C. Schreiber, B.H. Min, A.V. Zeiger and J.F. Lang, J. Pharmacol. Exp. Therap., 159, 372-78 (1968).
47. J. Riviere, Cah. Coll. Med. Hop. Paris, 9, 591-6 (1968).
48. B.W. Horrom, U. Biermacher and Kao Hwang, Am. Chem. Soc. Abstracts, April, 1968, pg. 10N.
49. E. Seeger, German Patent 1,265,751 (1968).
50. J.R. Boissier, J. Hirz, C. Dumont and A. Geardin, Ann. Pharm. Franc., 26, 215-26 (1968).
51. J.B. Hester, A.H. Tang, H.H. Keasling and W. Veldkamp, J. Med. Chem., 11, 101 (1968).
52. D.M. Gallant, M.P. Bishop, G. Bishop and L. O'Meallie, Curr. Therap. Res., 9, 579 (1967).
53. D.A. Cahal, Brit. Med. J., 2, 316 (1969).
54. F.J. Prime, Brit. Med. J., 3, 177 (1969).
55. W. Stuhlinger, G. Raberger and O. Kraupp, Naunyn Schmiedeberg Arch. Pharmakol., 264, 314-15 (1969).
56. O.Kraupp, W. Stuhlinger, G. Raberger and K. Turnheim, Naunyn Schmiedeberg Arch. Pharmakol., 264, 389-405 (1969).

57. H.P. Gurtner, M. Gertsch, C. Salzmann, M. Scherrer, P. Stucki, and F. Wyss., Schweiz. Med. Wschr., 98, 1695-1707 (1968).
58. Harmjanz, Gahl, Stender, Fabel and Deicher, Deutsch. Med. Wschr., 93, 2351 (1968).
59. E. Lang, E.J. Haupt, J.A. Koehler and J. Schmidt, Muench. Med. Wschr., 111, 405-12 (1969).
60. H. Schwingshackl, H. Amor and F. Dienstl, Deutsch Med. Wschr., 94, 639-45 (1969).
61. W. Schweizer, Praxis, 58, 701-02 (1969).
62. Anonymous, Med. Mschr., 23, 49-52 (1969).
63. R.E. Bagdon, H.A. Hartman and R.J. VanRyzin, Fed. Proc., 27, 598 (1968).
64. E.A. DeFelice, S. Bronstein and A. Cohen, Curr. Therap. Res., 11, 256-62 (1969).
65. A. Krese and J. Kisela, Ther. Hung., 16, 89-90 (1968).
66. G. Henkel, W.D. Henkel and G. Satzinger, Ger. Patent 1,271,118 (1968).
67. R.B. Moffett, J. Med. Chem., 11, 1251-2 (1968).
68. O. Thoma, G. Ludwig, H. Koppe and K. Zeile, Gr. Brit. Patent 1,109,364 (1968).
69. W.L. Dewey, M.H. Malone, L.S. Harris and I. Botton, Toxicol. Appl. Pharmacol., 12, 115-31 (1968).
70. M. Russek, J.A.F. Stevenson and G.J. Mogenson, Can. J. Physiol. Pharmacol., 46, 635-8 (1968).
71. J.R. Beaton and P.S. Vehara, Can. J. Physiol. Pharmacol., 47, 291-94 (1969).
72. A. Kekwick and G.L.S. Pawan, Lancet, 2, 198-99 (1968).
73. G.A. Bray and T.F. Gallagher, Jr., Lancet, 2, 1066-67 (1968).
74. J.H. Hutton and A.P. Creticos, Ill. Med. J., 132, 693-5 (1967).
75. H.A. Gusman, Am. J. Clin. Nutr., 22, 686-95 (1969).
76. M.J. Albrink, Am. J. Clin. Nutr., 22, 681-85 (1969).
77. A.A.H. Lawson, A.M.C. Gibson and P. Peattie, Brit. Med. J., 2, 246 (1969).
78. H. Bittnerova, J. Masek and R. Roth, Ges. Im. Med., 24, 139-42 (1969).
79. J. Pedersen and E.S. Olesen, Acta Endocrinol., 57, 683-8 (1968).
80. T.B. Vree, A. Th. J.M. Muskens and J.M. vanRossum, J. Pharm. Pharmacol., 21, 774-75 (1969).
81. R. Clark, Toxicol. Appl. Pharmacol., 15, 212-15 (1969).
82. J.M. vanRossum and F. Simons, Psychopharmacologia, 14, 248-54 (1969).

SECTION II - Pharmacodynamic Agents

Editor: Barry M. Bloom, Pfizer Inc., Groton, Connecticut

Chapter 6. Antihypertensive Drugs

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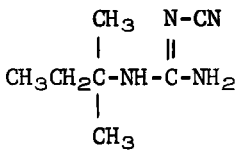
Most of the new compounds reported to show hypotensive or antihypertensive activity have received only preliminary study and none is clearly outstanding as a new lead.

Preliminary clinical reports on two compounds previously studied in animals indicate that each may represent a mechanistically new approach to the treatment of hypertension.

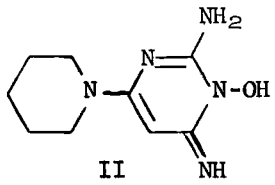
An excellent review of the chemical work on antihypertensives from 1962-1968 appeared.[1]

New Clinical Studies:



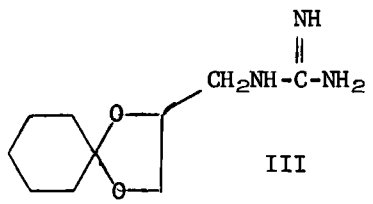
I

Guancydine (I) - Preliminary clinical studies[2] using oral doses of 250-750 mg. showed good reduction in blood pressure and a marked fall in peripheral resistance. Cardiac output increased. No tolerance or significant inhibition of sympathetic reflexes was observed. Reserpine prevented an increase in heart rate and diuretics potentiated the blood pressure fall.[3] Guancydine had previously been shown to inhibit angiotensin responses in normal (but not nephrectomized) animals.[4] A possibly unique mode of action is indicated.



II

PDP (II) - A clinical study of this direct vasodilator (15-80 mg., p.o.) in combination with propranolol to reduce reflex increases in heart rate was reported.[5] No orthostatic side effects were seen, and propranolol potentiated the hypotensive effect of PDP. Some electrolyte retention was seen.



III

Guanadrel (U 28288D, CL1388R) (III) - Preliminary clinical reports[6] using daily oral doses up to 400 mg. indicated good blood pressure response. Chlorthalidone potentiated the effect. Side effects were minimal. It appears to be similar in action to guanethidine.

Continuing Clinical Studies

Many studies of mechanisms of action and comparisons of clinically active compounds (particularly clonidine, guanethidine, debrisoquin, and methyldopa) were reported. No firm conclusions in addition to those reported last year can be drawn.

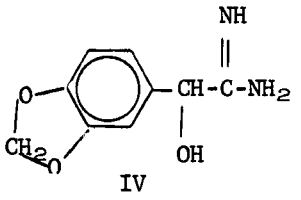
β-Blocking Agents - The value of propranolol in treatment of hypertension remains unclear. Mechanism studies have not indicated an action other than β-blockade.[7,8]

A clinical trial of alprenelol (1-(o-allylphenoxy)-3-isopropylamino-2-propanol) showed blood pressure reductions comparable to propranolol, However, alprenelol does not reduce peripheral resistance (as does propranolol) because of a β-sympathomimetic component of action.[9]

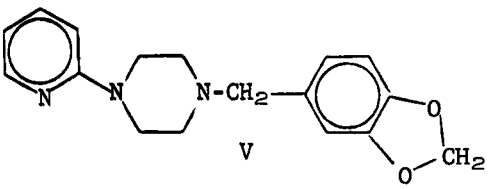
Prostaglandins - The hypotensive action of prostaglandins continues to be of interest as a possible basis for the development of a new type of antihypertensive agent. Arterial infusion of PGE_2 ($\sim 10^{-6}$ M.) reduced the amount of norepinephrine released by nerve stimulation. Such inhibition was suggested as a possible mechanism for its hypotensive effect.[10]

A proposal was made that the ability of the lungs to inactivate PGA_2 as well as to form angiotensin I may represent a mechanism of pulmonary control of blood pressure.[11]

New Compounds



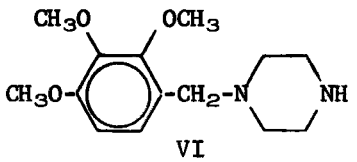
IV



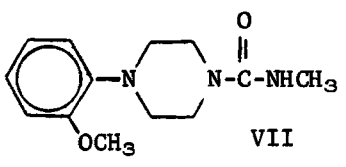
V

LL-1418 (IV) reduced both arterial pressure and peripheral resistance in conscious rats, dogs and rabbits.[12] The main activity was said to be CNS sedation and depression of sympathetic tone.

Trivastal, E-495, (V) is primarily a vasodilator causing selective increase in femoral blood flow while decreasing splanchnic flow. It caused a reduction in blood pressure, heart rate and respiration which was inhibited by vagotomy.[13]



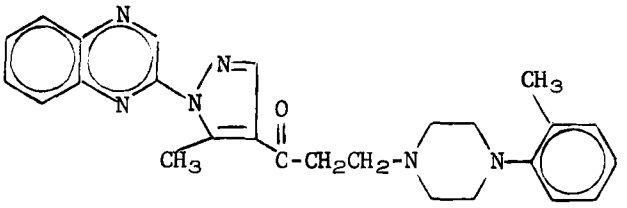
VI



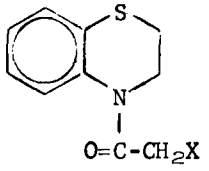
VII

Compound VI (USVP-D177) was orally active in reducing blood pressure of neurogenic hypertensive dogs. The effect appeared to be a combination of vasodilation and reduction in cardiac output.[14]

Of a series of 28 piperazines exemplified by VII, and corresponding thiourea derivatives, 11 showed hypotensive activity in rats.[15]



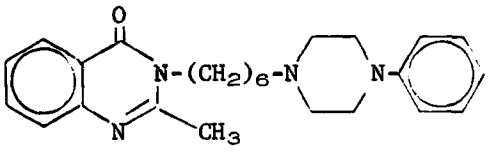
VIII



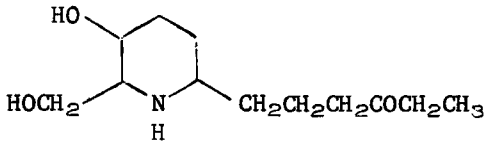
IX X= Cl or N_3

Compound VIII was the most active of a series of pyrazoles in reducing blood pressure in animals.[16] The epinephrine response was reversed but that of norepinephrine was not affected. There was no ganglionic blockade.

The dihydrobenzothiazines (IX) and their 1,1 dioxides reduced the blood pressure of cats without inhibition of the carotid occlusion response.[17]



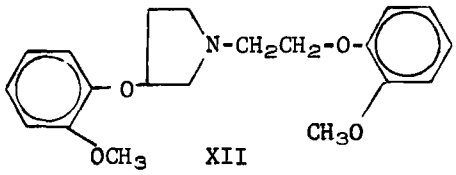
X



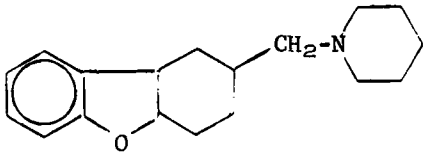
XI

The quinazolone X produced a long lasting fall in rat and dog blood pressure at i.v. doses as low as 0.1 mg./kg. In a series of related compounds hypotensive activity correlated in general with in vitro antiadrenergic potency.[18]

Prosopine (XI), isolated from African mimosa, showed hypotensive and vasodilatory activity, but produced marked gastric irritation in humans.[19]



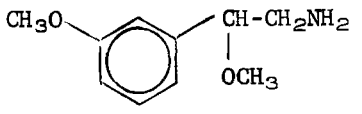
XII



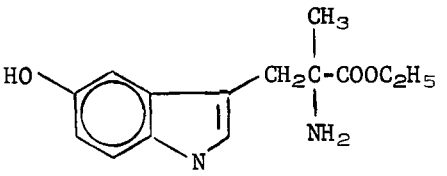
XIII

Compound XII was the most active of a series of methoxyphenoxypyrrolidines which reduced blood pressure in anesthetized cats by inhibition of sympathetic tone.[20]

The hypotensive effect of XIII in cats appeared to be due to a β-sympatholytic action.[21]



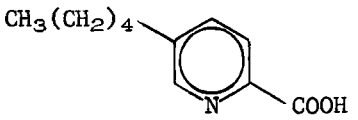
XIV



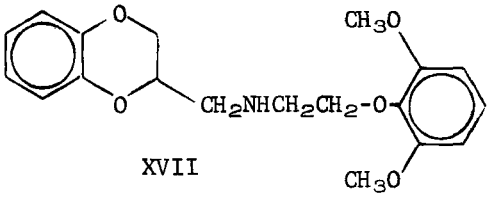
XV

The amine XIV reduced blood pressure in anesthetized cats. It was not a direct vasodilator and responses to norepinephrine and ganglion stimulation were increased.[22] A central inhibition of sympathetic tone is indicated.

The hypotensive action of XV in hypertensive rats was almost completely blocked by the decarboxylase inhibitor Ro 4-4602. A possible central action was indicated, but a "false transmitter" mechanism could not be ruled out.[23]



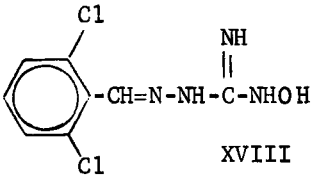
XVI



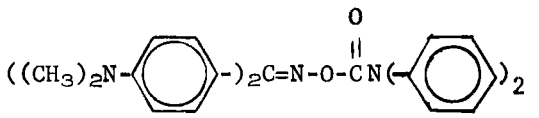
XVII

In a series of 5-n-alkylpicolinic acids, in vitro β-hydroxylase inhibiting activity was maximum with the n-pentyl analog (XVI). This analog was also the most potent in reducing blood pressure in anesthetized rabbits, but with longer chain analogs the two activities did not correlate.[24]

Activities in a series related to the adrenolytic compound XVII were found to show considerable structural specificity.[25] XVII is being studied as a hypotensive agent.



XVIII

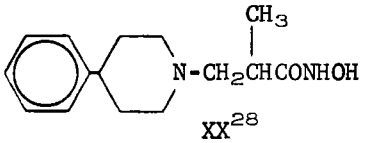


XIX

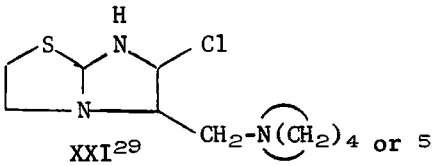
The hydroxamic acid XVIII lowered blood pressure and heart rate in renal hypertensive dogs and anesthetized cats. In spinal cats it showed only a pressor action.[26]

Compound XIX produced a hypotensive effect in rats presumably by a ganglioplegic action.[27] Diethylamino analogs were inactive.

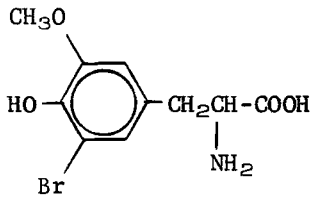
The following structures have been reported to produce hypotensive effects in animals but detailed data were not given.



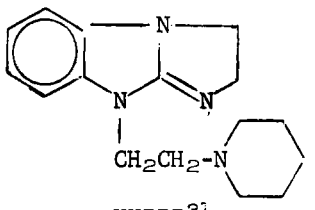
XX[28]



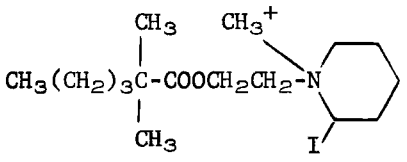
XXI[29]



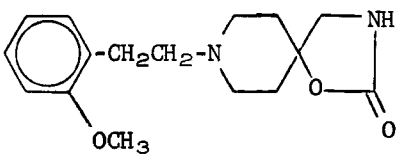
XXII[30]



XXIII[31]



XXIV[32]



XXV[33]

References

1. O. Schier and A. Marxer, Progress in Drug Research, 13, 101 (1969).
2. E. Freis and J. Hammer, Med. Ann. of Dist. of Col., 38, 69 (1969).
3. J. Hammer and E. Freis, Circ., 40 (Supp. III), 100 (1969).
4. J. Cummings and J. Grace, Jr., Pharmacologist, 11 (2), 270 (1969).
5. E. Gilmore, J. Weil and C. Chidsey, Circ., 40 (Supp. III), 89 (1969).
6. D. Bloomfield and J. Cangiano, Curr. Ther. Res., 11, 727 (1969).
7. C. Dollery, J. Patterson and M. Connelly, Clin. Pharmacol. Ther., 10, 765 (1969).
8. B. Prichard, Am. Heart J., 79, 128 (1970).
9. G. Tibblin and B. Ablad, Acta. Med. Scand., 186, 451 (1969).
10. P. Hedqvist, Acta Physiol. Scand., 77, (Supp. 330), 59 (1969).
11. P. Piper, J. Vane and J. Wyllie, Nature, 225, 600 (1970).
12. J. Duteil, M. Sautai, J. Montagne, J. Pessonier, R. Gombert and E. Assous, Therapie, 24, 711 (1969).
13. M. Laubie and H. Schmitt, Therapie, 24, 543 (1969).
14. J. Mirsky, J. Glassman, T. Southerland and B. Jandhyala, Pharmacologist, 11 (2), (1969).
15. R. Prasad and K. Tietje, J. Med. Chem., 12, 551 (1969).
16. V. Arya, R. Grewal, C. Kaul, S. Chate, D. Mehta and T. George, J. Pharm. Sci., 58, 432 (1969).
17. R. Prasad, J. Med. Chem., 12, 291 (1969).

18. S. Hayao, H. Havera and W. Strycher, J. Med. Chem., 12, 937 (1969).
19. P. Bourrinet and A. Quevauviller, Ann. Pharm. France, 26, 787 (1969).
20. R. Duncan, Jr., G. Helsley, W. Welstead, Jr., and B. Franco, J. Med. Chem., 12, 442 (1969).
21. M. Descamps, J. van der Elst, F. Binon, M. Colot, R. Charlier and F. Chaillet, Chim. Ther., 4, 251 (1969).
22. R. Howe, E. Young and A. Ainley, J. Med. Chem., 12, 998 (1969).
23. R. Tabei, S. Spector, W. Louis and A. Sjoerdsma, European J. Pharmacol., 7, 39 (1969).
24. H. Suda, T. Takeuchi, T. Nagatsu, M. Matsuzaki, I. Matsumoto and H. Umezawa, Chem. Pharm. Bull. Japan, 17, 2377 (1969).
25. P. Green, M. Shapiro and C. Wilson, J. Med. Chem., 12, 327 (1969).
26. L. Boyajy, R. Manning, R. MacIntosh, K. Fix, M. Herzig and J. Trapold, Fed. Proc., 27, 783 (1969).
27. M. Kochnar, B. Williams and J. Fan, J. Pharm. Sci., 58, 1382 (1969).
28. R. Coutts, K. Midah and K. Prasad, J. Med. Chem., 12, 940 (1969).
29. J. Paolini and L. Lindvay, ibid., 12, 1031 (1969).
30. P. Crooij and J. Eliaers, J. Chem. Soc., 559 (1969).
31. A. Simonov, A. Belous, V. Anisimova and S. Ivanosky, Khim. Farmatseut. Zh., 7 (1969).
32. A. Lespagnol, C. Lespagnol, J. Bonvarlet, J. Cazin and M. Cazin, Chim. Therap., 4, 103 (1969).
33. G. Regnier, R. Canevari, J. LeDourarec, M. Laubie and J. Duhault, ibid., 4, 185 (1969).

Chapter 7. Drugs for the Therapy of Pulmonary Disorders

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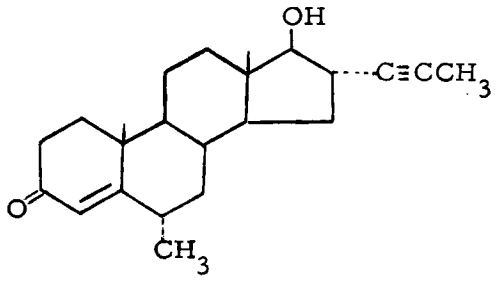
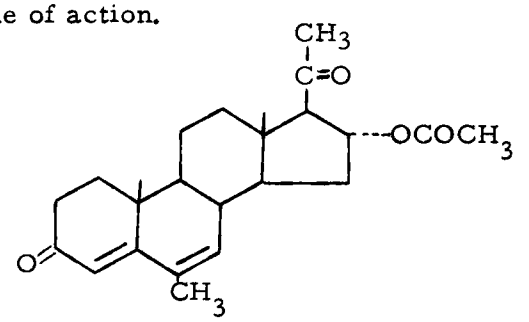
Introduction - This chapter will review two groups of drugs used for the relief of various manifestations of lung diseases, namely, the bronchodilators to terminate or prevent an asthmatic attack, and the pulmonary vasodilators for the treatment of pulmonary hypertension. It should be noted first of all that in the treatment of respiratory diseases the approach is symptomatic rather than etiotropic. The causes of bronchospasm and pulmonary hypertension are difficult to control. Disease processes as such, for example, viral infections of the respiratory system, pulmonary emphysema and pulmonary arteriosclerosis, cannot be treated successfully by drugs, so that one has to resort to treatment of symptoms.

Progestational Hormones - New drugs relating to the etiology of lung disease may soon be forthcoming because of the promising leads uncovered in the investigation of progestational agents in experimental pulmonary emphysema. Until recently, attempts to develop a method for testing drugs against the development of experimental emphysema have been unsuccessful. In 1968, a method became available consisting of ligation of the trachea and intratracheal injection of trypsin or phytohemagglutinin in immature rats[1]. The concurrent injection of progesterone prevented the appearance of emphysema. More recently, the oral administration of dimethisterone or megestrol, both orally effective progestagens, prevented the appearance of emphysema[2]. The mechanism for the effect of these steroids has not been identified. A bronchodilator effect has been excluded, but an influence of the hormone on the reaction of pulmonary tissue to injury has not been verified as the mode of action.



Dimethisterone



Megestrol

Bronchodilator Drugs - Most of the bronchodilators introduced in recent years belong to the sympathomimetic class. The pharmacology of sympathomimetic drugs in general, and of bronchodilators in particular, has been

reviewed in a monograph[3] that is appearing concurrently with this volume. The mode of action of sympathomimetics is essentially that of relaxation of bronchial smooth muscles brought about by direct stimulation of adrenergic β-receptors.

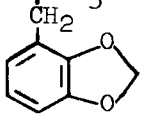
The stimulation of adrenergic β-receptors is accomplished by three types of sympathomimetics: the epinephrine-type, which directly stimulates β-receptors in the airways as well as α-receptors in the blood vessels; the ephedrine-type, which indirectly stimulate β- and α-receptors by releasing catecholamines; and the isoproterenol-type, which directly stimulate β-receptors but not α-receptors.

The drugs comprising the β stimulant group are listed in Table 1. Besides isoproterenol, there are eleven others, almost all of which have additional features which render them more beneficial than isoproterenol. Some are effective by the oral route, some have a prolonged duration of action, and some influence the cardiovascular system to a lesser extent than the bronchopulmonary system. The adrenergic β-receptors in the heart and bronchial airways are equally sensitive to isoproterenol. It has been possible to stimulate the bronchial receptors to a greater degree than the cardiac receptors. However, it has not been possible to develop a sympathomimetic drug that stimulates the heart more strongly than the bronchial and vascular smooth muscles. The development of drugs that act selectively on the pulmonary blood vessels is discussed in the following sections.

Biogenic substances which could be involved in the etiology of pulmonary hypertension - Before discussing pulmonary vasodilators, it seems desirable to consider some of the more recent data relating to biogenic substances which can induce pulmonary vasoconstriction. This information will be helpful in understanding the complexities of attempting to elucidate the mechanism of pulmonary hypertension. Pulmonary hypertension is a serious clinical problem and it can occur without apparent organic cause, as is the case in primary pulmonary hypertension, or secondary to organic changes, as is the case in pulmonary hypertension associated with pulmonary emphysema, bronchial asthma, myocardial septal defects or cor pulmonale. The search for pulmonary vasodilators is complicated by the lack of mechanistic knowledge about pulmonary hypertension and the lack of adequate test methodology. Most procedures currently employed require a great deal of experimental surgery on the anesthetized animal. Anesthesia itself may increase arteriovenous shunting, further complicating the situation. Most importantly, it must be remembered that the pulmonary vasculature possesses a great amount of passive distensibility. Therefore, in the resting or the anesthetized animal, a pulmonary vasodilator may have little or no effect on the pulmonary arterial pressure. It

TABLE 1

Bronchodilators That Stimulate Adrenergic β Receptors

Drug Name (Year Introduced)	Chemical Structure						Bronchodilator Dosage		
	R	R_1	R_2	R_3	R_4	R_5	oral mg.	inhaled	Subcutaneous mg.
Isoproterenol (1940)	3-OH	4-OH	-OH	-H	-H	$-CH(CH_3)_2$		1%	0.25
Etafedrine (1930)	-H	-H	-OH	$-CH_3$	$-CH_3$	$-C_2H_5$	50		
p-Hydroxyephedrine (1939)	-H	4-OH	-OH	$-CH_3$	-H	$-CH_3$	50		
Methoxyphenamine (1940)	-H	$2-OCH_3$	-H	$-CH_3$	-H	$-CH_3$	50-100		
Ethylnorepinephrine (1944)	3-OH	4-OH	-OH	$-C_2H_5$	-H	-H			1-2
Isoetharine (1950)	3-OH	4-OH	-OH	$-C_2H_5$	-H	$-CH(CH_3)_2$		1%	2
Protokylol (1954)	3-OH	4-OH	-OH	-H	-H		2-4	1%	
Clorprenaline (1956)	2-Cl	-H	-OH	-H	-H	$-CH(CH_3)_2$	20		
Dioxethedrine (1960)	3-OH	4-OH	-OH	$-CH_3$	-H	$-C_2H_5$	20		
Metaproterenol (1961)	3-OH	5-OH	-OH	-H	-H	$-CH(CH_3)_2$	10-20	2%	
Soterenol (1964)*	$3-NHSO_2CH_3$	4-OH	-OH	-H	-H	$-CH(CH_3)_2$			
Salbutamol (1968)**	$3-CH_2OH$	4-OH	-OH	-H	-H	$-C(CH_3)_3$		1%	

*Clinical dose not yet determined[4].
**Clinical dose not yet determined[5].

appears necessary, a priori, to utilize a method in which the pulmonary arterioles are constricted.

Several biogenic substances have been implicated as mediators in the induction of pulmonary vasoconstriction. Since the pulmonary vasculature is innervated by the sympathetic nervous system[6], it would appear that catecholamines released by the nerve endings are involved in neurogenic vasoconstriction. However, there does seem to be a difference in the response of the pulmonary vasculature to endogenously released norepinephrine as compared with the response to that administered exogenously. It has recently been determined that stimulation of the sympathetic nervous system in the anesthetized dog causes stiffening of the large pulmonary arteries, which favorably affects the distribution of blood within the lungs. Exogenously administered norepinephrine seemed to act mainly on the pulmonary arterioles, leading to an increase in pulmonary vascular resistance[7]. The exogenous injection of epinephrine has also been shown to produce pulmonary vasoconstriction[8]. It is felt that activation of the sympathetic nervous system occurs reflexly as a result of hypoxic and acidotic stimulation of the carotid and aortic chemoreceptors[9, 10]. Although hypoxia can induce pulmonary vasoconstriction by reflex mechanisms, there is a great deal of evidence indicating that systemic hypoxia can cause pulmonary vasoconstriction directly or by the release of a non-catecholamine mediator[11, 12, 13, 14]. Alveolar hypoxia also produces pulmonary vasoconstriction, probably by the release of a mediator substance[15, 16].

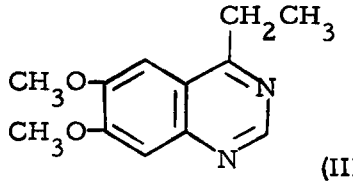
Serotonin has been found to exert an intense vasoconstrictor effect on the pulmonary circulation[8, 17, 18]. Although the pulmonary vasoconstrictor effect of serotonin can be demonstrated easily by pharmacological methods, its physiological and pathophysiological roles have not been unequivocally demonstrated. In fact, the infusion of serotonin (1.85 - 5.5 mcg/kg/min) into the pulmonary artery of seven human subjects produced a definite rise in pulmonary artery pressure in only one instance[19]. Most of the serotonin found in whole blood is stored in platelets. This would imply that, in the process of platelet aggregation to form a thrombus, serotonin would be available for release[20]. Pulmonary vasoconstriction does occur as a result of pulmonary thrombosis. A further indication that serotonin could be implicated in the thrombosis-induced pulmonary vasoconstriction is the finding that 82 per cent of serotonin can be released from platelets by thrombin in five minutes[21].

Other endogenously formed pulmonary vasoconstrictors have been implicated in the development of pulmonary hypertension. Histamine has been extensively studied with results indicating that it may play a role in the genesis of hypoxia-induced pulmonary hypertension[22, 23]. Angiotensin has been found to produce a pulmonary vasoconstrictor effect in the

isolated rat lung preparation[24]. Prostaglandin $F_{2\alpha}$ has been shown to be the predominant prostaglandin in the lung[25]. It is worthwhile to note that this compound exerts an intense constrictor effect on the pulmonary arteries and veins[26]. Prostaglandin $F_{2\alpha}$ can be readily synthesized by the guinea-pig lung from arachidonic acid[27].

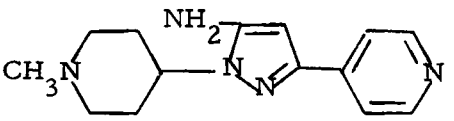
Pulmonary Vasodilators - It appears that relatively little effort is being devoted to the search for pulmonary vasodilators, perhaps because of the reasons discussed above. There is certainly no lack of therapeutic need. During the past year, however, no new compounds have been introduced solely for the use of dilating the pulmonary vasculature.

A comprehensive evaluation of drugs affecting the pulmonary circulation was undertaken a few years ago[28]. In the two volumes published at that time, much space was devoted to compounds then used for their pulmonary vasodilator ability, such as isoproterenol and aminophylline. Although no new compounds have found their way into the physicians' armamentarium as pulmonary vasodilators since that time, several compounds have received a good deal of investigative attention for that particular therapeutic effect. One compound is a quinazoline, MJ-1988 (III), bearing the generic name of quazodine. This compound was found to possess the triad of effects which are considered desirable in treating cor pulmonale, i. e., cardiac stimulation, pulmonary vasodilatation and bronchodilatation[29]. Moreover, quazodine was found to be of particular interest because it was well absorbed from the gastrointestinal tract and because the cardiac stimulant and bronchodilator properties could not be antagonized by agents known to block adrenergic β-receptors[30].



(III)

6,7-dimethoxy-4-ethylquinazoline

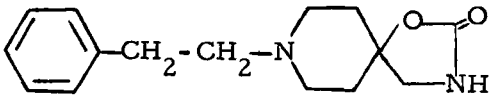


(IV)

4-[5-amino-3-(4-pyridyl)-1-pyrazolyl]-1-methylpiperidine (Ciba 31531-Ba)

Another compound which has received much attention recently is Ciba 31531-Ba. This product (IV) was reported to have a vasodilator effect on the pulmonary circulation in the open chest, right heart-bypass cat with fixed cardiac output. It was suggested that the compound caused pulmonary vasodilatation by exerting a direct effect on the vasculature[31]. Moreover, Ciba 31531-Ba was found to reduce experimental pulmonary arterial hypertension in dogs when administered intravenously in doses of 1-2 mg/kg[32]. In the same article it was reported that, when a dose of 400 mg q. i. d. was administered orally to 16 patients with chronic cor

pulmonale, (IV) produced a reduction in the incidence of dyspnea and cyanosis and an increase in O_2 saturation and physical capacity. Recently (IV) was studied in seven patients with pulmonary hypertension[33]. The compound was reported to decrease the total pulmonary resistance and the pulmonary arterial pressure, and to increase minute volume. It appeared that most patients developed a reduction in arterial pCO_2 and an increase in pH.



(V)

A more recent compound, fenspiride (V), possesses a multiplicity of pharmacology, most of which appear desirable in the treatment of pulmonary disease[34, 35]. Besides a bronchodilator effect, a weak anti-inflammatory effect, an inhibitory effect on sensitivity reactions, and an α-adrenergic blocking effect, the compound was reported to decrease the pulmonary artery pressure in the anesthetized dog. This decrease in pulmonary artery pressure may be due to a non-specific vasodilatory activity. Preliminary clinical trials indicate that the compound is effective in the treatment of asthma[36]. Clinical studies evaluating the effect of (V) in pulmonary hypertension have not been reported.

The nonapeptide, bradykinin, has been reported to induce pulmonary vasodilatation in the foetal lamb[37]. It was suggested that bradykinin might be responsible for the reactive hyperemia which ensues after ischemia in unventilated foetal lungs. The vasoactive polypeptide, substance P, reduced the pulmonary arterial pressure of anesthetized dogs, as measured by pulmonary artery catheterization[38]. The effect was transient in nature and thought to be the result of direct action on the pulmonary vasculature.

In summary, it appears that only compounds exhibiting a generalized vasodilator or adrenergic β-stimulating effect are being evaluated for their ability to produce pulmonary vasodilatation. This approach may lead to the detection of agents which are efficacious but the over-all systemic activity can be predicted to lead to undesirable side effects. It seems that the time has come for us to elevate our goals and strive for compounds with greater selectivity for pulmonary structures. It is teleologically important that we strive for the ideal in our search for a pulmonary vasodilator, i. e., a specific agent existing solely for the pulmonary vasculature with little or no effect on the systemic vasculature.

References

1. H. Ito and D. M. Aviado, J. Pharmacol. Exp. Therap., 161, 197 (1968)
2. D. M. Aviado and G. R. McKinney, Pharmacol. Research Communications, 1, 283 (1969).
3. D. M. Aviado, Sympathomimetic Drugs, Springfield, Charles C. Thomas, 1970, pp. 1-565.
4. B. M. Cohn, Current Therap. Res., 11, 713 (1969).
5. K. N. V. Palmer and M. L. Diament, Brit. Med. J., 1, 31 (1969).
6. O. Larsell and R. S. Dow, Amer. J. Anat., 52, 125 (1933).
7. R. H. Ingram, Jr., L. P. Szidon and A. P. Fishman, Circ. Res., 26, 249 (1970).
8. A. M. Rudolph and E. M. Scarpelli, Amer. J. Physiol., 206, 1201-1206 (1964).
9. D. M. Aviado, J. S. Ling and C. F. Schmidt, Amer. J. Physiol., 189, 253-262 (1957).
10. I. deB. Daly and M. deB. Daly, J. Physiol., (London) 148, 201-219 (1959).
11. G. R. Barer and J. R. McCurrie, ibid., 191, 82P-83P (1967).
12. A. S. Abraham, R. B. Cole, I. D. Green, R. B. Hedworth-Whitty, S. W. Clark and J. M. Bishop, Circ. Res., 24, 51-60 (1969).
13. E. D. Silove and R. F. Grover, J. Clin. Invest., 47, 274-285 (1968).
14. E. D. Silove, T. Inoue and R. F. Grover, J. Appl. Physiol., 24, 355-365 (1968).
15. I. deB. Daly and C. Hebb, Pulmonary and Bronchial Vascular Systems, E. Arnold (Publishers) Ltd. London, 1966, pp. 249-288.
16. K. H. Nilsen and A. Hauge, Acta Physiol. Scand., 73, 111-120 (1968).
17. R. F. Grover, S. K. Olson and S. G. Blount, Clin. Res., 6, 62 (1958).
18. K. Yoshitake, Jap. J. Circ., 31, 853-871 (1967).
19. P. Harris, H. W. Fritts and A. Cournand, Circulation, 21, 1134 (1960).
20. J. W. Sullenberger, W. G. Anlyan and W. T. Weaver, Surgery, 46, 22 (1959).
21. J. R. Gaintner, D. P. Jackson and E. W. Maynert, Bull. Johns Hopkins Hosp., 111, 185 (1962).
22. A. Hauge, Acta Physiol. Scand., 72, 33-44 (1968).
23. A. Hauge, Circ. Res., 22, 371-392 (1968).
24. L. Joubert, L. Tetreault and P. Biron, Rev. Canad. Biol., 25, 293-294 (1966).
25. E. Anggard, Prostaglandins in the Lung: Studies on Their Occurrence, Biosynthesis, Metabolism and Biological Properties, Published by the Depts. of Chemistry and Pharmacology, Karolinska Inst., Stockholm (1966), p. 13.
26. A. Human, J. Pharmacol. Exp. Therap., 165, 267-273 (1969).
27. E. Anggard and B. Samuelsson, J. Biol. Chem., 240, 3518-3521 (1965).

28. D. M. Aviado, The Lung Circulation, Pergamon Press, New York (1965) vol. I and II.
29. D. M. Aviado, L. E. Folle and J. Pisanty, J. Pharmacol. Exp. Therap., 155, 76-83 (1967).
30. P. M. Lish, R. H. Cox, K. W. Dungan and S. I. Robbins, Pharmacologist, 6, 181 (1964).
31. H. Brunner, K. Eichenberger, M. Meier, M. Wilhelm and P. Schmidt, Experientia, 22, 208-209 (1966).
32. A. J. Georgopoulos, G. Mplatzas and K. Tountos, Cardiologia Suppl. 1, 42, 23-31 (1966).
33. K. Jedlicka, S. Daum, L. Nikodymova and J. Stiksa, Cas. Lek. Cesk., 108, 629-633 (1969).
34. J. C. Le Douarec, J. Duhault and M. Laubie, Arzneimittel-Forschung, 19, 1263 (1969).
35. G. Regnier, R. Covevari, J. C. Le Douarec, M. Laubie and J. Duhault, Chimica Therap., 4, 185 (1969).
36. J. A. M. A., 209, 1615 (1969).
37. A. G. M. Campbell, G. S. Dawes, A. P. Fishman, A. I. Hyman and A. M. Perks, J. Physiol. (London), 195, 83-96 (1968).
38. G. M. Maxwell, Brit. J. Pharmacol. Chemother., 32, 514-522 (1968).

Chapter 8. Antianginal Drugs

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Until a very few years ago, an annual report with this title could be considered overly optimistic; no drug other than the short-acting nitrates (or nitrites) had yet been shown clearly to be of value in the treatment of angina pectoris -- and nitrates are more than a hundred years old. The formidable difficulties that the clinical investigator of antianginal drugs must face have recently been authoritatively summarized by a Panel on Cardiovascular Drugs of the National Research Council.[1] The consequences of this situation for drug research are all too clear; until a particular kind of pharmacological or biochemical action can be correlated with proven clinical efficacy, there is little rational basis for seeking new agents.[2] It appears, moreover, that even the one clinically validated lead may have been misleading. The nitrates are undoubtedly coronary vasodilators, and the search for more potent and longer acting coronary vasodilators has until recently been the prevailing theme of drug research in this area. More potent vasodilators have indeed been found, but they have been generally disappointing clinically; and it now seems that coronary vasodilation is not likely to be the major basis, or at least not the sole basis, for the usefulness of nitrates in angina.

This gloomy picture has brightened considerably, however, with the advent of the "β-blocker" drugs -- those agents that block the action of catecholamines at the adrenergic β-receptors. Work on the antianginal effects of these agents has accelerated greatly in the two years since the last report, and they will be a major theme of the present report.

Reviews - A number of general reviews on angina therapy have appeared in the past two years.[3-8] Non-drug approaches will not be discussed here, but a few key references might be cited.[3,9-12] A particularly useful review on the metabolism of the heart in health and disease has appeared.[13] More specialized reviews will be cited later.

Nitrates - Glyceryl trinitrate and the other short-acting nitrates (or nitrites), commonly used sublingually to terminate the acute attack, are still the only drugs of unquestioned value in angina. Evidence in regard to the longer-acting preparations, used orally for prophylaxis, is conflicting, but the general consensus seems to be that they are of limited value.[14] In the only study[15] that came close to meeting the stringent criteria of the NRC Panel,[1] the long-acting nitrates tried were without benefit. Evidence is conflicting also for the utility of various sustained release or aerosol formulations of organic nitrates, and tolerance may develop.[16-18]

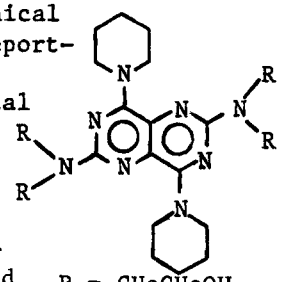
The mode of action of the nitrates is still not completely clear; recent work on this important question has been reviewed.[2,14,19] The coronary vasodilation seen in animals and normal man is transient,[19] and may be quickly followed by reduced coronary blood flow (CBF).[20] There

are several compelling reasons for believing that this cannot be the major basis for the antianginal effect. Myocardial hypoxia (ischemia), accepted as the cause of the anginal attack, is the most powerful stimulus known for vasodilation,[2] and the inadequate response that leads to angina is believed usually to result from inability of sclerosed arteries to respond even to such a powerful stimulus. Under these circumstances, it is not surprising that coronary vasodilators, even those which are more potent than the nitrates in animals or normal man, are generally ineffective in angina patients. A much more convincing case can be made for reduction by nitrates of the work load of the heart, and therefore its demand for oxygen, by virtue of their well-known ability to decrease peripheral resistance and venous return to the heart.[3,14,19] In this hypothesis, it must be assumed that the compensatory increase in heart rate and the reduced CBF that are often seen are not of sufficient magnitude to negate the beneficial effect on heart work and oxygen demand of the lowered peripheral resistance. There is some evidence to support a net reduction in left ventricular work.[19,21]

The evidence for a beneficial effect of nitrates on collateral circulation in the heart has been summarized.[14] And the interesting proposal has been made that nitrates, by dilating mainly the large coronary arteries, improve the blood supply to ischemic areas, while other coronary vasodilators, by acting primarily on smaller vessels, could actually divert blood from the ischemic regions.[22]

Coronary Vasodilators - Despite the foregoing, interest remains high, particularly in Europe, in drugs that are usually categorized as coronary vasodilators. Although most of these were selected on the basis of coronary vasodilator activity in animals, and although many have shown such an action in normal humans, it is clear that these agents often have other pharmacological actions that could contribute to an antianginal effect.[23] Clinical efficacy has not been demonstrated unequivocally for any of these drugs, but several have been widely used in Europe, and, based largely on uncontrolled studies, reported to be useful in some proportion of angina patients. Several appear to have been studied in the U.S., but only one (dipyridamole) appears to be marketed here for antianginal use. No comprehensive review has been found, but several papers discuss more than one of these drugs.[24-26]

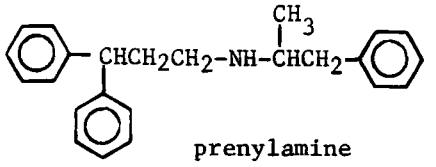
Dipyridamole, one of the first modern drugs of this type, still continues to be extensively studied, at both the clinical and laboratory levels. Clinical efficacy has been reported for the drug alone,[27,28] or in combination with a tranquilizer (oxazepam).[29,30] The consensus of medical opinion, however, seems to be that antianginal efficacy has not been proved.[2,31,32] Recent hemodynamic studies in animals[33,34] and man[35] confirm the marked increases in CBF previously reported with dipyridamole, and reinforce the conclusion that coronary vasodilation, at least when measured as increased CBF, is not a sufficient basis for antianginal



R = CH_2CH_2OH
dipyridamole

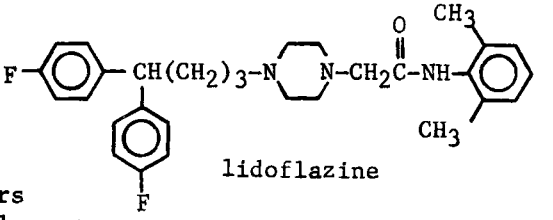
activity. As noted above,[22] the exact site of drug action in the coronary vascular tree may be important. Fam and his collaborators have provided additional evidence that dipyridamole acts primarily on the small resistance vessels, while the nitrates dilate the large arteries.[36] Adenosine is a powerful vasodilator, and although its role in the autoregulation of the coronary circulation[37] is not entirely clear, it is often stated that dipyridamole acts by potentiating adenosine;[24] little new evidence has been found.

Generally favorable results have been reported in recent clinical trials of prenylamine,[38,39] but caution has been urged in its use, since it can apparently lead to fatal cardiac arrest.[2] The drug increases CBF in animals, but has a number of other cardiovascular actions, as well as sedative properties, and a reserpine-like effect on catecholamine stores. It is by no means clear which of these are important in accounting for its antianginal activity, but an indirect (or β-) sympathomimetic action appears to have been ruled out.[40]



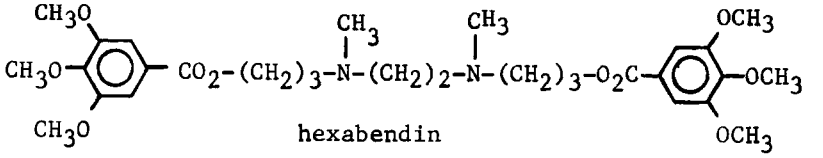
prenylamine

Lidoflazine has been the subject of a long-term clinical trial, followed by an unusually careful, placebo-controlled study.[41] The drug was found to be significantly more effective than placebo, and to be well tolerated. Patients taken off drug during this trial deteriorated only slightly, and it was thought that this might reflect development of collateral coronary circulation, as has been reported in animals. Pharmacologically, this agent appears to be similar to dipyridamole, but longer acting. Additional evidence has been presented that lidoflazine, like dipyridamole, has an adenosine-sparing or potentiating action.[42]



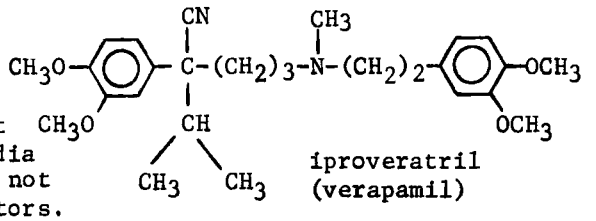
lidoflazine

Another of the newer drugs, hexabendin (hexobendin), is reported also to have an adenosine-sparing or potentiating effect.[25] Based on work in dogs, however, it has been proposed that an essential component in its action is induction of a primary metabolic acidosis, with secondary effects on coronary resistance.[43] As is usual in early, uncontrolled clinical trials with drugs of this type, antianginal efficacy of varying degree has been reported for hexabendin.[44,45] Headache appeared to be the most common side-effect.



hexabendin

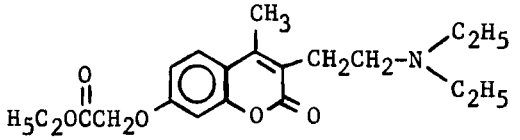
Recent British clinical studies with iproveratril (verapamil) have been discussed.[46] Despite considerable further work, the mode of action of this interesting agent remains an enigma.[47,48] It appears to share some of the actions of the nitrates, e.g. increased CBF and lowered peripheral resistance.[47] Unlike that produced by the nitrates, however, the increased CBF results from dilation of the small resistance vessels. Like the β-blocker drugs, iproveratril decreases heart rate in animals and has antiarrhythmic activity; but unlike these drugs, it does not always reduce (and may increase) the force of myocardial contractions.[47,48] Moreover, it does not prevent exercise-induced tachycardia in healthy volunteers,[49] and does not appear to block pulmonary β-receptors.



iproveratril (verapamil)

It has been suggested that antianginal activity results from α-adrenergic blockade,[50] or that it results from myocardial depressant activity.[49]

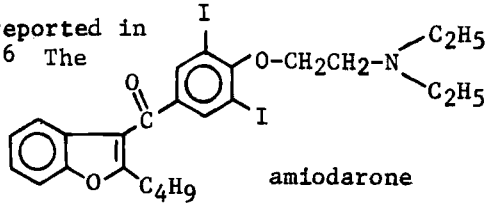
Fleckenstein[51] has developed the attractive hypothesis that iproveratril, some of its analogs (e.g. D600, Knoll), and prenylamine interfere with excitation-contraction coupling by complexing calcium ion, which is required by the myofibrillar ATP-ase. Blockade of β-receptors would produce a similar result, but in a different way, by preventing catecholamine/Ca activation of the ATP-ase. Calcium ion did indeed reverse the depressant effects of iproveratril (and the other drugs mentioned) on isolated papillary muscle of the guinea pig; but the inconsistent effects of iproveratril on contractile force in whole animals, noted above, detract somewhat from this otherwise interesting hypothesis.



carbochromen (chromonar)

A few additional clinical trials have been reported with carbochromen (chromonar),[52] and increased CBF has been seen with this drug in heart patients.[53] Improved collateral circulation was also reported in one patient, after six-month treatment with carbochromen.[54]

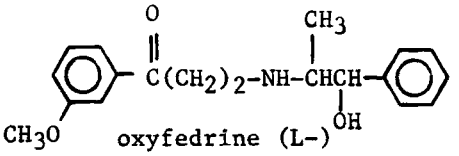
Favorable results in angina were reported in several recent trials of amiodarone.[55,56] The blocking activity of this drug on both α- and β-adrenergic receptors, reported earlier by Charlier,[57] has been confirmed.[58]



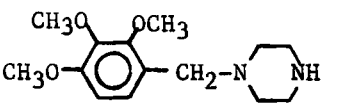
amiodarone

Little of substance has been found in the recent literature on visnadine or pyridinol carbamate. Early papers have appeared on a number of new agents, but space restrictions preclude giving more than structures

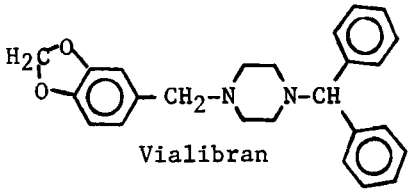
and key references. These include oxyfedrine[59,60] and analogs,[61] trimetazidine,[62] Vialibran,[63] heptaminol and derivatives,[64] etafenon,[65] bencyclan,[66] N 1113,[67] imolamine,[68] and 730 CERM.[69]



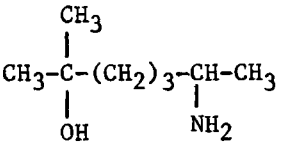
oxyfedrine (L-)



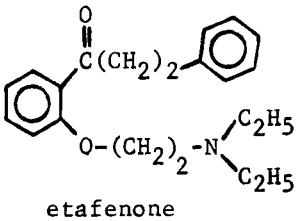
trimetazidine



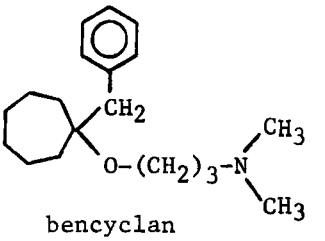
Vialibran



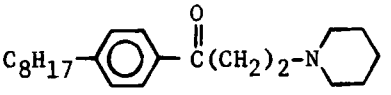
heptaminol



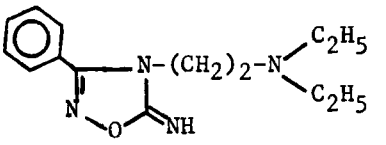
etafenone



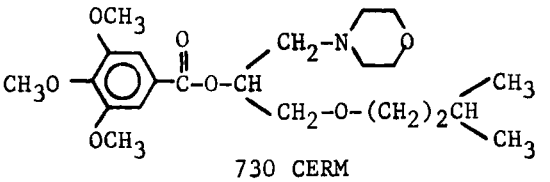
bencyclan



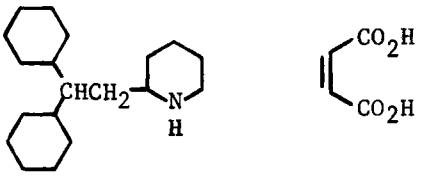
N-1113



imolamine



730 CERM



perhexiline maleate

One new drug that appears to be of more than average interest is perhexiline maleate.[70] Like an earlier analog, hexadiline (hexadylamine), this agent increases both coronary and peripheral blood flow. In contrast to the nitrates, however, doses that markedly lower blood pressure do not

increase heart rate or reduce CBF, apparently because there is little effect on venous return to the heart. Although perhexilene diminishes exercise-induced tachycardia, it has no effect on resting heart rate, and does not appear to block β-receptors;[71] on the contrary it is reported to have bronchodilator properties in man.[72] Early clinical trials suggest that it may prove to be useful in angina.[70,73]

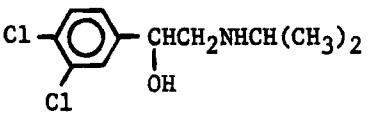
β-Blockers - It has been estimated that almost a thousand papers have appeared on these agents, in the few years since they were introduced into clinical practice. Fortunately, several good reviews on their use in angina are available,[74-76] and these should be consulted for additional references. The review by Fitzgerald[74] is a particularly lucid and orderly introduction to the subject. The pharmacology of these drugs has been reviewed recently,[77] but the latest comprehensive discussions of structure-activity relationships appear to be those of Ariens[78] and Biel and Lum.[79]

Of the earliest β-blockers, dichloroisoproterenol (DCI) was considered to have too much β-sympathomimetic (β-agonist) activity for clinical use; and pronethalol was withdrawn from clinical trial because of carcinogenicity in rodents. Propranolol was therefore the first drug of this class to be thoroughly studied clinically. Although it is not yet approved in the U.S. for use in angina, the consensus of medical opinion appears to be favorable, i.e. the drug is of definite value in a significant proportion of angina patients.[75,76]

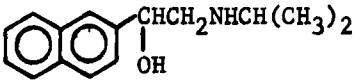
The rationale for use of β-blockers in angina is straightforward. In contrast to those of other organs, the adrenergic receptors in the heart are almost all of the β-type, including those that affect rate (chronotropic) and force of contraction (inotropic). Angina is often precipitated by emotional and other factors that involve excessive activity of the sympathetic nervous system and/or increased levels of circulating catecholamines. Blockade of the β-receptors in the heart prevents the increased contractile work and oxygen demand that result from stimulation of chronotropic and inotropic receptors by the catecholamines. Unfortunately there are other consequences of β-blockade, and other pharmacological actions of the existing agents, which may not be appropriate to the anginal state.

Most serious, perhaps, is the precipitation of heart failure in patients who are dependent on sympathetic drive to maintain sufficient cardiac output. Fitzgerald argues that this can readily be avoided by proper digitalization of the patient, and use of diuretics.[74] Because of the reduced cardiac output, blood pressure can fall, and this may be undesirable in some cases; and the reduction in resting heart rate may also be undesirable in some patients. Stimulation of β-receptors in smooth muscle, as in most tissues other than the heart, leads to relaxation; the tone of vascular and pulmonary smooth muscle is therefore increased by blockade of the β-receptors. The consequences are decreased CBF and increased coronary arterio-venous oxygen difference, which may be undesirable in angina; and increased airways resistance, which can provoke dyspnea or aggravate bronchial asthma. These effects are usually attrib-

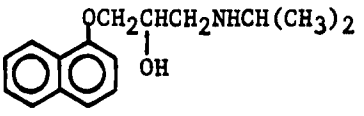
uted to α-responses unmasked by β-blockade, but the reduced CBF may result in part from decreased oxygen demand by the heart. The reduced CBF can apparently be countered to some extent by nitrates; and the tachycardia sometimes seen with nitrates should in turn be suppressed by the negative chronotropic effect of the β-blocker. Management of angina with a combination of propranolol and isosorbide dinitrate continues to be strongly advocated by Russek,[80,81] and converts are appearing.[82]



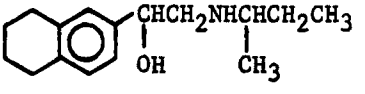
dichloroisoproterenol (DCI)



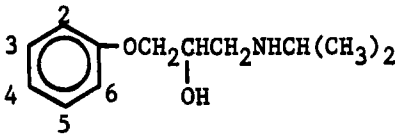
pronethalol



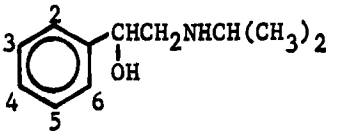
propranolol



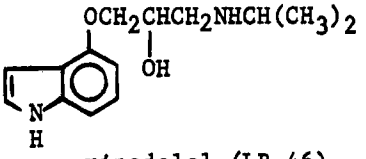
butidrine



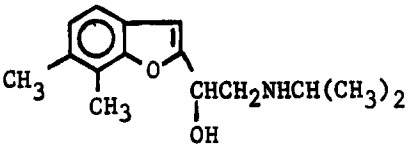
Kö 592 (ICI 45,763): 3-CH_3
alprenolol (H 56/28): 2-$CH_2CH{=}CH_2$
oxprenolol (BA 39,089): 2-$OCH_2CH{=}CH_2$
practolol (ICI 50,172): 4-$NHCOCH_3$
DU 21,445: 2-SCH_3



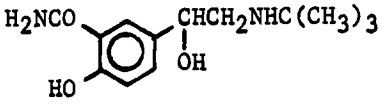
INPEA: 4-NO_2
sotalol (MJ 1999): 4-$NHSO_2CH_3$
H 13/57: 3,4-di-CH_3



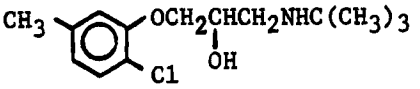
prinodolol (LB 46)



Ro 3/3528



AH 3474



KL 255

As noted above, DCI exhibits marked β-agonist activity, i.e. it has intrinsic sympathomimetic activity (ISA). The agents introduced more recently are weaker agonists, and propranolol, for example, is virtually devoid of ISA. It has been proposed[83] that a certain degree of ISA might be desirable in avoiding precipitation of heart failure or bronchospasm. However, blockers with varying degrees of ISA, e.g. alprenolol, oxprenolol, pronethalol, and INPEA all appear to have precipitated heart failure.[74]

Another important action of most β-blockers is that referred to by Fitzgerald[74] as their membrane activity. This is reflected in local anesthetic activity, and a quinidine-like, depressant action on the heart. Unlike that of quinidine, however, the myocardial depressant action (MDA) of β-blockers is not reversed by acetylcholine.[84] This non-specific MDA, which is most prominent with propranolol and the more lipophilic drugs, appears to result from membrane stabilization and reduced conduction velocity of nerve impulses. The possibility that the membrane activity of these drugs plays a part in some of their antiarrhythmic effects has been reviewed[85] (outside the scope of this report). An important question, especially for future research in this area, is whether the non-specific MDA plays a part in the antianginal activity of these drugs.

The doses of propranolol required to elicit membrane activity are typically about four times the β-blocking dose. Principally because of the high doses of propranolol required in some patients for antianginal effects, it has been suggested[86,87] that the hemodynamic and clinical actions of the drug are in fact due to a direct, non-adrenergic depressant action on the heart. However, the (+) isomer of propranolol (dexpropranolol), which has membrane activity equal to that of the racemate but is virtually devoid of β-blocking activity, fails to show the hemodynamic effects of the racemic drug in dogs.[88] Similar results have been reported from human studies with dexpropranolol,[89] and with the dextro isomer of alprenolol as well.[90] The wide individual variations in effective clinical doses could be explained by variations in sympathetic activity and catecholamine concentrations at the receptors, since the known agents are all competitive blockers. Isoproterenol challenge experiments are not directly relevant, and it does not yet appear possible to assess this possibility at the clinical level. Fitzgerald[74] has suggested an alternative explanation for the wide variations in clinical doses of propranolol, based on individual differences in drug metabolism. Further clinical experience with β-blockers such as INPEA, sotalol, and practolol, which are essentially free of membrane activity, should allow a final decision on the importance of non-specific MDA to the antianginal effects of β-blockers. A very recent clinical pharmacology experiment with sotalol and propranolol[91] suggests that they may prove to be equally effective in angina, and that β-blocking activity, rather than non-specific MDA, is probably the basis for clinical activity.

The therapeutic value of the newer drugs in the structure table remains to be established, and the literature is still quite limited on some of them. Several have already been mentioned, and others are discussed in the reviews cited; but key references can be given for a few of the newest

agents. Prinodolol (LB 46) is reported to be a more potent β-blocker than propranolol, with weak ISA and little membrane activity.[92] Ro 3/3528 was 1/10-1/15 as potent as propranolol in blocking isoproterenol-induced tachycardia in man.[93] In vivo, AH 3474 is apparently comparable in potency to propranolol, but it is much less potent on isolated tissues;[94] this was attributed to its relative polarity and freedom from membrane effects. This agent is also devoid of ISA. In animal studies, KL 255 appeared to be somewhat more potent than propranolol when given intravenously, but not when given orally.[95] In normal humans, oral absorption also appeared to be relatively poor;[96] no published clinical studies in angina were found.

Of particular importance to future research in this area is the recent emergence of β-blockers with greater tissue selectivity. Practolol (ICI 50,172), a somewhat less potent β-blocker than propranolol, has very little effect on bronchial receptors.[97,98] If the early clinical reports[99,100] of efficacy in angina are confirmed, it may prove to be the β-blocker of choice for patients with bronchopulmonary problems. There was also a suggestion of myocardial selectivity in the human studies with KL 255.[96] A likely explanation for the tissue selectivity of practolol can be found in the reclassification of adrenergic receptors by Lands and coworkers.[101] Responses of various animal tissues to a series of sympathomimetic amines revealed, for example, significant differences between the β-receptors of heart (β-1) and smooth muscle (β-2). This sub-division of β-receptors has been further supported by work with some new β-sympathomimetic drugs.[102]

Also in accord with this classification are some interesting recent experiments in dogs with practolol.[103] CBF was actually increased at doses of the drug that blocked the positive chronotropic and inotropic effects of isoproterenol, suggesting that the rate/force receptors in the myocardium are distinctly different from the β-receptors in the coronary vasculature. The authors attribute this to the β-sympathomimetic component (ISA) of practolol, but selectivity for the myocardial β-receptors would appear to be a sufficient explanation. There are already suggestions that β-receptors may need to be further subdivided,[104] and that the β-1 receptors of the myocardium may not be homogeneous.[102] Specifically, there is evidence for differentiation of the rate (chronotropic) and force (inotropic) receptors.[102,105] Implications to the development of still more selective β-blocking drugs are clear. What is not yet so clear is whether further selectivity of this kind will be valuable in the treatment of angina.

References

1. Circulation, 41, 149 (1970).
2. K. I. Melville, Pharmacology for Physicians, 4, No. 3, 1 (1970).
3. D. T. Mason, J. F. Spann Jr., R. Zelis, and E. A. Amsterdam, New Eng. J. Med., 281, 1225 (1969).
4. M. B. Matthews and W. P. G. Turck, Practitioner, 202, 203 (1969).
5. Brit. Med. J., 2, 134 (1969).

6. Brit. Med. J., 2, 677 (1969).
7. W. Meesmann, Therapiewoche, 18, 632 (1968).
8. K. Donat, Med. Klin. (Munich), 63, 1793 (1968).
9. W. C. Sheldon, F. M. Sones Jr., E. K. Shirey, D. J. G. Fergusson, R. G. Favaloro, and D. B. Effler, Circulation, 39, Suppl. 1, 61 (1969).
10. S. E. Epstein, G. D. Beiser, R. E. Goldstein, M. Stampfer, A. S. Wechsler, G. Glick, and E. Braunwald, New Eng. J. Med., 280, 971 (1969).
11. E. A. Amsterdam, S. Wolfson, and R. Gorlin, Ann. Intern. Med., 68, 1151 (1968).
12. H. N. Hultgren and E. J. Hurley, Advan. Intern. Med., 14, 107 (1968).
13. L. H. Opie, Amer. Heart J., 76, 685 (1968); ibid., 77, 100, 383 (1969).
14. A. B. Weisse and T. J. Regan in Current Status of Drugs in Cardiovascular Disease, ed. C. K. Friedberg, p. 216, Grune and Stratton, New York, 1969.
15. S. L. Cole, H. Kay, and G. C. Griffith, Circulation, 15, 405 (1957).
16. G. Burke, Geriatrics, 22, 168 (1967).
17. C. Spier, Clin. Med., 76, 23 (1969).
18. W. Kaiser and H. Klepzig, Med. Welt, No. 33-34, 1786 (1968).
19. Brit. Med. J., 4, 252 (1969).
20. R. P. Carson, W. S. Wilson, M. J. Nemiroff, and W. J. Weber, Amer. Heart J., 77, 579 (1969).
21. M. Arborelius, Jr., H. Lecerof, A. Malm, and R. O. Malmborg, Brit. Heart J., 30, 407 (1968).
22. M. M. Winbury, B. B. Howe, and M. A. Hefner, J. Pharmacol. Exp. Ther., 168, 70 (1969).
23. D. Palm, Naunyn-Schmiedeberg's Arch. Pharmakol. Exp. Pathol., 263, 159 (1969).
24. R. Charlier, Schweiz. Apoth. Ztg., 107, 523 (1969).
25. O. Kraupp, Naunyn-Schmiedeberg's Arch. Pharmakol. Exp. Pathol., 263, 144 (1969).
26. P. Duchene-Marullaz, D. Cosnier, and J. Grimald, Thérapie, 24, 617 (1969).
27. M. C. Becker, M. Weiner, A. Shapiro, and A. Gibson, J. Amer. Med. Ass., 203, 56 (1968).
28. M. Wirecki, J. Chronic Dis., 20, 139 (1967).
29. R. Klever, Therapiewoche, 18, 837 (1968).
30. J. Kandziora, Med. Welt, No. 39, 2314 (1967).
31. Brit. Med. J., 2, 562 (1969).
32. Med. Lett. Drugs Ther., 8, 27 (1966).
33. R. Kadatz and W. Diederen, Aerztl. Forsch., 21, 51 (1967).
34. J. Simaan and G. Fawaz, Cardiovasc. Res. 2, 68 (1968).
35. P. J. Schollmeyer and D. Jeschke, Klin. Wochenschr., 47, 42 (1969).
36. W. M. Fam and M. McGregor, Circ. Res., 22, 649 (1968).
37. R. M. Berne, Physiol. Rev., 44, 1 (1964).
38. J. B. Stoker, Brit. J. Clin. Pract., 22, 384 (1968).
39. M. S. Belle, J. Fl. Med. Ass., 54, 116 (1967).
40. E. Lindner, Arzneim.-Forsch., 19, 15 (1969).
41. L. K. Verhaeghe, ibid., 19, 1842 (1969).
42. A. H. M. Jageneau and W. K. A. Schaper, Nature (London), 221, 184 (1969).
43. O. Kraupp, W. Grossmann, W. Stühlinger, and G. Raberger, Arzneim.-

Forsch., 18, 1067 (1968).
44. J. Dockweiler, ibid., 18, 1081 (1968).
45. W. Zimmermann, ibid., 18, 1112 (1968).
46. Drug Ther. Bull., 7, 19 (1969).
47. G. Ross and C. Jorgensen, Amer. Heart J., 76, 74 (1968).
48. H. Haas, Arzneim.-Forsch., 18, 89 (1968).
49. R. G. Shanks, Lancet, 2, 560 (1967).
50. L. Garvey, H. E. Shister, and K. I. Melville, Can. Med. Ass. J., 98, 113 (1968).
51. A. Fleckenstein, H. J. Döring, and H. Kammermeier, Klin. Wochenschr., 46, 343 (1968).
52. G. Friese and D. Eisenbarth, Med. Welt, No. 36, 1896 (1968).
53. W. Rudolf, L. Meixner, and H. J. Künzig, Klin. Wochenschr., 45, 333 (1967).
54. A. Düx and A. Schaede, Deut. Med. Wochenschr., 94, 1349 (1969).
55. A. Leutenegger and E. Lüthy, Schweiz. Med. Wochenschr., 98, 2020 (1968).
56. M. Vastesaeger, P. Gillot, and G. Rasson, Ned. Tijdschr. Geneesk., 112, 1313 (1968).
57. R. Charlier, G. Deltour, A. Baudine, and F. Chaillet, ibid., 112, 1312 (1968).
58. P. R. Moret, D. Boufas, and P. C. Fournet, Schweiz. Med. Wochenschr., 99, 1090 (1969).
59. E. Stampfer, Wien. Klin. Wochenschr., 81, 288 (1969).
60. J. Beyreder, Wien. Med. Wochenschr., 118, 717 (1968).
61. K. Thiele, K. Posselt, and W. von Bebenburg, Arzneim.-Forsch., 18, 1263 (1968).
62. T. N. Mehrotra and E. T. Bassadone, Brit. J. Clin. Pract., 21, 553 (1967).
63. S. A. Cantor, Med. J. Aust., 2, 983 (1967).
64. P. Duchene-Marullaz, D. Cosnier, S. Szabo, and F. Mollat, Thérapie, 23, 383 (1968).
65. O. Hohl, D. Bernhardt, G. E. Kentschke, and R. Metzger, Therapiewoche, 19, 1653 (1969).
66. G. Lamm and J. Hollo, Ther. Hung., 15, 95 (1967).
67. E. Karpati, L. Domok, and L. Szporny, Arzneim.-Forsch., 19, 1011 (1969).
68. W. Christmann, Med. Welt, No. 46, 2559 (1968).
69. J. Pernod and J. Kermarec, Thérapie, 24, 675 (1969).
70. T. Winsor, Clin. Pharmacol. Ther., 11, 85 (1970).
71. Y. W. Cho, S. Matsuo, and D. M. Aviado, Fed. Proc., Fed. Amer. Soc. Exp. Biol., 28, 671 (1969).
72. O. Feinsilver, D. M. Aviado, and Y. W. Cho, Clin. Res., 17, 414 (1969).
73. I. Hirshleifer, Curr. Ther. Res. Clin. Exp., 11, 99 (1969).
74. J. D. Fitzgerald, Clin. Pharmacol. Ther., 10, 292 (1969).
75. C. T. Dollery, J. W. Paterson, and M. E. Conolly, ibid., 10, 765 (1969).
76. W. C. Elliott and J. M. Stone, in Current Status of Drugs in Cardiovascular Disease, ed. C. K. Friedberg, p. 227, Grune and Stratton, New York, 1969.
77. B. R. Lucchesi and L. S. Whitsitt, ibid., p. 20, 1969.
78. E. J. Ariens, Ann. N. Y. Acad. Sci., 139, 606 (1967).
79. J. H. Biel and B. K. B. Lum, Progr. Drug. Res., ed. E. Jucker, 10, 46, Birkhäuser Verlag, Basel, 1966.
80. H. I. Russek, J. Amer. Med. Ass., 204, 528 (1968).

81. H. I. Russek, Geriatrics, 24, 81 (1969).
82. D. C. Harrison, New Eng. J. Med., 280, 895 (1969).
83. B. Ablad, Acta Pharmacol. Toxicol., 25, Suppl. 2, 5 (1967).
84. J. V. Levy and V. Richards, J. Pharmacol. Exp. Ther., 150, 361 (1965).
85. D. Gibson and E. Sowton, in Current Status of Drugs in Cardiovascular Disease, ed. C. K. Friedberg, p. 160, Grune and Stratton, New York, 1969.
86. W. C. Elliott and R. Gorlin, Mod. Conc. Cardiovasc. Dis., 35, 111 (1966).
87. Brit. Med. J., 1, 37, 331 (1969).
88. A. M. Barrett, J. Pharm. Pharmacol., 21, 241 (1969).
89. A. G. Wilson, O. G. Brooke, H. J. Lloyd, and B. F. Robinson, Brit. Med. J., 4, 399 (1969).
90. P. Bjorntorp, Acta Med. Scand., 184, 259 (1969).
91. J. M. Kofi Ekue, D. C. Lowe, and R. G. Shanks, Brit. J. Pharmacol., 38, 546 (1970).
92. J.-F. Giudicelli, H. Schmitt, and J. R. Boissier, J. Pharmacol. Exp. Ther., 168, 116 (1969).
93. R. C. Hill and P. Turner, Brit. J. Pharmacol., 32, 663 (1968).
94. C. H. Blackburn, L. J. Byrne, V. A. Cullum, J. B. Farmer, and G. P. Levy, J. Pharm. Pharmacol., 21, 488 (1969).
95. H. Jacobi and R. Fontaine, Fortschr. Med., 85, 749 (1967).
96. E. Waterloh, H. F. Rittel, and E. Leide, Arzneim.-Forsch., 19, 153, 330, 1704 (1969).
97. D. Dunlop and R. G. Shanks, Brit. J. Pharmacol., 32, 201 (1968).
98. A. G. MacDonald and R. S. McNeil, Brit. J. Anaesth., 40, 508 (1968).
99. D. Gibson and E. Sowton, Brit. Med. J., 1, 213 (1968).
100. N. H. Areskog and L. Adolfsson, ibid., 2, 601 (1969).
101. A. M. Lands, A. Arnold, J. P. McAuliff, F. P. Luduena, and T. G. Brown, Jr., Nature (London), 214, 597 (1967).
102. J. B. Farmer, I. Kennedy, G. P. Levy, and R. J. Marshall, J. Pharm. Pharmacol., 22, 61 (1970).
103. W. D. Bussman, M. Rauh, and H. P. Krayenbuehl, Amer. Heart J., 79, 347 (1970).
104. J. B. Farmer and G. P. Levy, J. Pharm. Pharmacol., 22, 145 (1970).
105. L. S. Cohen, G. F. Vastagh, E. McLaughlin, and J. H. Mitchell, Circulation, 40, Suppl. 3, 60 (1969).

Section III - Chemotherapeutic Agents

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Chapter 9: Antibiotics and Related Compounds

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General - The clinical investigation of new antimicrobial agents constitutes a formidable challenge demanding broad knowledge of antibiotic action in the management of the patient with infectious disease[1]. General reviews dealing with the proper selection and use of antibiotics[2], the elaboration of certain principles in their use[3], and the comparative merits of newer antibiotics[4], have appeared in 1969. A treatise of antibiotic knowledge has been published in the German language[5].

Increased efforts are being directed towards control of gram-negative organisms[6a-c]. The announcement of therapeutic efficacy of the aminoglycoside, gentamicin[7], together with that of carbenicillin[8], cephalosporins[9] and others[2,4], have raised the hope that such control is attainable.

There is need for more effective suppression of Neisseria gonorrhoeae infections[4], particularly in areas of high incidence[10] or those involving strains of reduced penicillin-sensitivity[11]. New agents recommended against this gonococcus are carbenicillin[12], doxycycline, minocycline[12,13] and others[12]. Drug resistant tuberculosis in Scandinavia and elsewhere is continuing to respond favorable to capreomycin, ethambutol, and rifampicin, in combinations[14].

Resistance to antibiotics - Resistance to antibiotic therapy, especially multiple-drug resistance mediated by transfer factors, remains of active concern. There is a growing awareness that antibiotic therapy of the patient and his illness needs to be considered in the light of newer knowledge bearing on the possible effects of such therapy on microbial ecology[15,16].

The study of soil and stool samples obtained from drug-free, "antibiotic-virgin" communities[17,18] revealed the presence of E. coli strains harboring R factors which mediate resistance to tetracycline[17,18] streptomycin[17,18], ampicillin[18] and chloramphenicol[18].

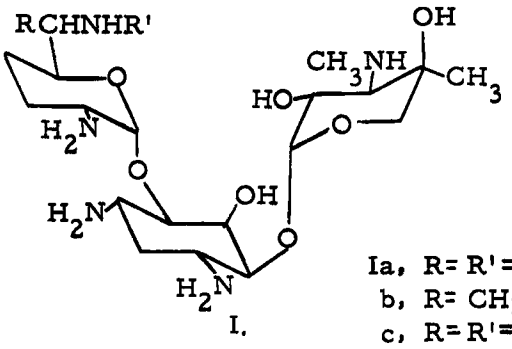
In vivo transmission of R factor has been demonstrated in mice[19], in weanling pigs and in the alimentary tract of a human being[20]. Transfer of drug resistance in vivo, however, was found to be a rare occurrence[20,21];

also, increased recipient ability for R factor was observed to be associated with "rough" cultures of reduced virulence[22]. These latter important observations[20-22] have prompted a novel interpretation of the role of antibiotics in animal feeds[23].

In further biochemical studies of transmissible resistance, enzymatic adenylation, known for streptomycin[7d, e, 24-26], was shown to be a pathway of inactivation also for the structurally related bluensomycin[7d] as well as for the seemingly unrelated spectinomycin (actinospectacin)[7d]. Another route of inactivation, enzymatic phosphorylation, known for kanamycin[26,27], neomycin[26,27] and others has been observed for streptomycin[7d,24]. A phosphorylase preparation capable of inactivating the above aminoglycoside antibiotics, did not effectively phosphorylate components of the gentamicin group[7d,e].

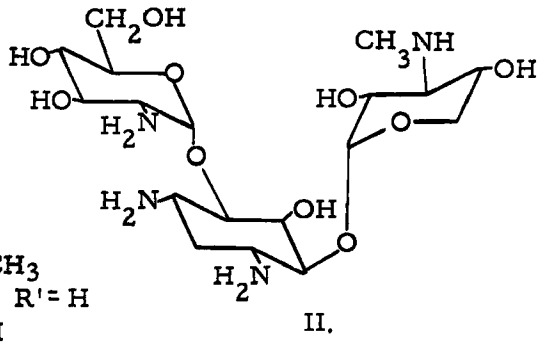
Mannosidostreptomycin, a biologically active analog, according to a recent report[28], is not adenylated by a kinase capable of inactivating streptomycin. Intriguingly, adenosine, as well as the C-nucleoside formycin, is a competitive inhibitor of ATP which serves as phosphate donor in the inactivation of kanamycin by a phosphorylase of P. aeruginosa[27].

Aminoglycosides - The recognition of the therapeutic qualities of gentamicin, reported in depth at an International Symposium in October, 1968[7], constitutes a major advance in the control of serious, life-threatening infections[7a] especially those due to gram-negative bacilli resistant to kanamycin and other aminoglycoside antibiotics[7f]. Microbiological studies with the clinically available form of gentamicin, a mixture of the three components gentamicin C_1, C_{1a} and C_2, (Ia, Ib and Ic) showed that among more than 1,000 clinical isolates of Pseudomonas in 21 separate groups, 94% were found to be sensitive to 10 μg/ml of the antibiotic (tube dilution method)[7g]. Favorable protective activity was noted also against E. coli and the Klebsiella-Enterobacter group, against Proteus and Neisseria gonorrhoeae, Serratia and other genera[7f,g]. Among strains of Staphylococcus aureus, 99% were found to be sensitive to 5 μg/ml of gentamicin or less[7g] but the antibiotic is not highly active against meningococci[7g].



I.

Ia, R= R'= CH_3
b, R= CH_3, R'= H
c, R= R'= H



II.

It has been noted that R factor resistance does not appear to play a major role with gentamicin[7h] (cf. Lack of phosphorylative inactivation of gentamicin C complex noted above[7e]).

Partition chromatographic methods effectively provided the three components C_1, C_{1a} and C_2 for structure determination[7k]; microbiological and pharmacological studies revealed a near-identity of antibacterial, pharmacokinetic, and toxicity characteristics of the individual components[7g].

Studies of the comparative nephro- and ototoxicity of aminoglycoside antibiotics generate assurance that in the effective clinical dose range of 1-3 mg/kg[7s] and with peak serum levels not above 12 μg/ml[7t], gentamicin does possess a favorable margin of safety in man.

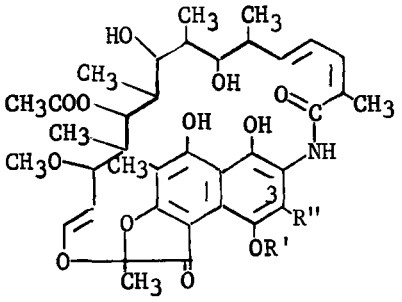
Utilizing mutants of S. fradiae, which do not synthesize neomycins B or C because of a blocked deoxystreptamine pathway, novel hybrid antibiotics, hybrimycins A_1 and A_2 or B_1 and B_2, were produced by feeding the related sub-units, streptamine and epi-streptamine, respectively, to growing cultures of these mutants[29].

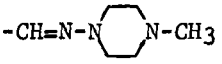
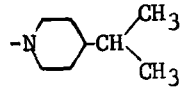
Ansa-macrolides - Extensive progress in the study and clinical use of rifampin (rifampicin) and other rifamycins is reflected in the more than a hundred publications dealing with their antibacterial[30-32], anti-viral (see Chapter 11), pharmacokinetic[30,33,34] and therapeutic qualities[14,35-39]. Characteristically, ansa-macrolides (ansa-mycins[?][40]), the rifamycins, streptovaricin, and tolypomycin, are thought to exert their antibacterial action through suppression of RNA-polymerase function. This mechanism of inhibition, however, was observed at one hundred times higher levels, with the structurally divergent antibiotic streptolydigin[41] and indications for an alternate or additional mechanism have been noted in inhibition studies of rifampin-resistant bacteria with semisynthetic rifamycin SV derivatives[42].

Micro-organisms highly sensitive to inhibition by rifampin, include penicillin-sensitive as well as penicillin-resistant S. aureus[30], D. pneumoniae[33], group A streptococci[33], H. influenzae[35], Proteus[30], M. tuberculosis in vitro[31] and in the mouse[31,32]. Plasma levels above 2 μg/ml were attained in man between two and four hours after oral administration of a 300 mg dose of rifampin[33]. A major portion of the drug is found in the bile[33,34] in the form of 25-desacetyl rifampin[34] with evidence of entero-hepatic circulation of this biologically active metabolite.

The growth of 50 different strains of Neisseria meningitidis in vitro was found to be inhibited by rifampin in concentrations of 1 μg/ml or less[37] and subsequently the antibiotic was shown to be uniquely effective in the eradication of these meningococci from asymptomatic carriers[37].

The powerful anti-mycobacterial activity[31,32] of rifampin (III), a leading drug[14] against tuberculosis in man[32,38], also manifests itself against M. leprae growing in the mouse foot-pad[39]. In man, rapid effective suppression of lepromatous leprosy was observed with daily 600 mg doses of rifampin administered for 20 weeks[39].



	R'	R''
III	H	
IV	H	
V	H	
VI	H	
VII	H	H
VIII	$-CH_2COOH$	H

Among a group of semi-synthetic derivatives, 3-piperidino- (IV), 3-homo-piperidino- (V), and 3-(4-isopropylpiperidino)-rifamycin SV (VI) were active not only against rifampin-sensitive strains of S. aureus and B. subtilis (MIC↿ 0.002-0.01 μg/ml)but also against rifampin-resistant strains (MIC↿ 1-10 μg/ml)[42]. Rifamycin SV (VII), a precursor of rifamycin B (VIII) fermentation, has been produced directly by growing cultures of a mutant strain of Streptomyces mediterranei[43]. Confirmation of structure and stereochemistry of tolypomycinone was obtained through X-ray analysis of the tri-m-bromobenzoate[44].

β-Lactam Antibiotics - A potentially predictive relationship between the sum of molar attraction constants, indicative of the relative degree of drug-receptor interaction, and observed in vivo antibacterial activities appears to exist for a group of substituted α-phenoxy-6-acylamidopenicillanic acids[45]. α-Sulfoaminobenzylpenicillin possesses in vitro anti-pseudomonas activity of the order of carbenicillin and is effective against P. aeruginosa infections in the urinary tract of the rat[46]. In clinical investigations 6-D-α-azidophenylacetamidopenicillanic acid, (azidocillin) was found to be as effective as penicillin V in the treatment of scarlet fever but super-infection with H. influenzae was observed only in cases treated with the latter antibiotic[47].

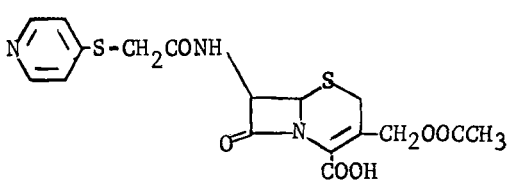
α-Carboxypenicillin, carbenicillin, is valued in the treatment of Pyocyaneus infections of the urinary tract[48], of Pseudomonas infections[49] in cancer patients[8] and, in combination with gentamicin, of Pseudomonas

pneumonia[7j]. On the other side, the emergence of resistant strains of Pseudomonas aeruginosa has been noted[49].

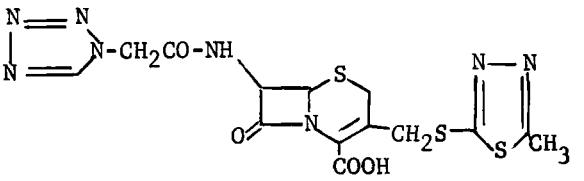
The immunochemical aspects of penicillin antibiotics have been reviewed in depth[50,51]. It has been suggested, on the basis of model reactions with thiol containing compounds, that the reaction of penicillenic acid with free SH groups of protein may be antigenically involved in penicillin allergy[52]. A penetrating study of bacterial resistance to penicillin and to cephalosporin antibiotics[53] has been made available.

Structure-activity relationships for a group of semi-synthetic phenylglycine derivatives of 7-aminodesacetoxycephalosporanic acids, including cephalexin, have been compared with those of the corresponding cephalosporins[54].

Among new semi-synthetic cephalosporin antibiotics BL-P 1322 (IX)[55], compared with cephalothin, is characterized by superior activity in vitro against D. pneumoniae and M. tuberculosis, while cefazolin (X)[56] has shown activity against clinically isolated strains of E. coli and K. pneumoniae which are strongly resistant to cephalothin and cephaloridine.



IX



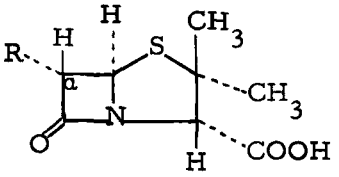
X

Cephaloridine has been found to be of value in chronically uremic patients undergoing serial dialysis in the absence of kidney function[57]. The successful use of this antibiotic in the treatment of a small number of patients with streptococcal endocarditis has been reported[58]. Forty-nine of eighty patients with acute as well as chronic urogenital infections, rapidly improved on an oral regimen of 500 mg. doses of cephaloglycin three times a day for fourteen days[59]. In a similar study involving 245 patients from 4 to 92 years of age, a high percentage of the acute urinary tract infections responded favorably to cephaloglycin[60]. In these studies of cephaloglycin, the most common complaint was diarrhea[59,60].

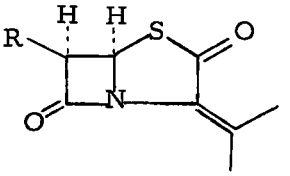
Cephalexin inhibits the in vitro growth of most gram-positive bacteria, including penicillin-resistant, but not methicillin-resistant S. aureus strains[61]; half of E. coli and one-third of Proteus clinical isolates were among the susceptible gram-negative cultures but isolates of P. aeruginosa and Serratia were highly resistant[61]. In the treatment of patients with urinary tract[9] and upper respiratory[62] infections, oral cephalexin has

been recognized as an effective, well-tolerated agent. Metabolic studies have established that in mice and rats, cephalexin is absorbed from the gastro-intestinal tract as the intact antibiotic[63]. Cephalexin is not metabolized in the body and is eliminated as unaltered antibiotic, primarily via the urine[63].

Accounts of chemical syntheses in the field of β-lactam antibiotics have appeared in book form[64]. Papers dealing with the epimerization of the α-carbon in the β-lactam ring afford an understanding of the requirements[65] and mechanism[66] of this conversion in both penicillins[67] (C-6) (XI) and cephalosporins[68] (C-7).



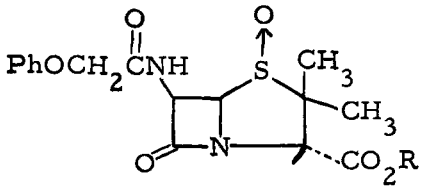
XI



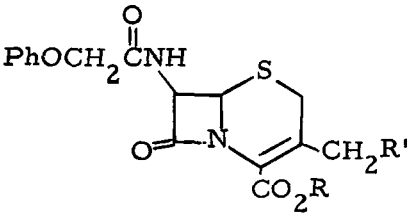
XII

A variety of unusual transformation products[69,70] has been produced from penicillins under acylating conditions. One of these, an anhydropenicillin (XII)[70], has now been reconverted to the parent antibiotic under mild conditions.

Considerable effort has been devoted to the determination of the configuration and conformation of the sulfoxides of β-lactam containing antibiotics[71-74]. These elegant studies, which shed further light on the mechanism of the ring expansion processes of penicillin sulfoxides (XIII), have been facilitated by the large anisotropic effects observed in the NMR spectra of these compounds[72,75]



XIII



XIV R'=H

XV R'=-OCCH$_3$ (O)

The metamorphosis of penicillin V sulfoxide ester (XIII) into a deacetoxy cephalosporin (XIV) has been reported in detail by Morin and coworkers[76]. The introduction of the acetoxy function into the 3-methyl group of XIV, generating a cephalosporanic acid (XV) has now been accomplished[77].

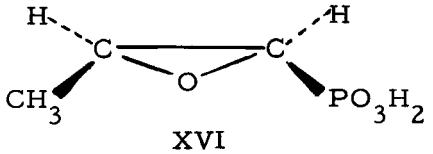
Coumermycin A_1 is highly effective against "methicillin-resistant" strains of S. aureus[78] in vitro and may have promise as an agent for the treatment of infections due to group A streptococci, pneumococci, and other organisms[79]. A new semi-synthetic derivative of coumermycin A_1, BL-C 43, was shown to be markedly superior to novobiocin, lincomycin, and erythromycin in oral therapy of S. aureus infections in the mouse[80].

7-Chloro-7-desoxylincomycin (clindamycin) is well suited for anti-infectious treatment of infants and children[81]. 7-Deoxy-7(R)- and -7(S)-thiolincomycin possessed slight antibacterial activity[82]. Microbial transformation of clindamycin produced clindamycin sulfoxide which had one fourth of the antibacterial activity of the parent antibiotic against S. lutea[83].

Macrolide antibiotics - Structural assignments have been made for spiramycin[84], in relationship to leucomycins and carbomycin, for cirramycin A_1[85] and the related tylosin[86], and for megalomycin A[87a,b], 11-O-β-D-rhodosaminyl-erythromycin C. Megalomycin is produced by Micronospora megalomiceia cultures. It is somewhat less active than erythromycin. Megalomycin when given orally is efficiently absorbed and well tolerated in dogs. Intricate studies[88], utilizing specialized NMR techniques[89] in an examination of erythronolide species, have resulted in an accurate portrayal of the conformation of these molecules in solution[88,89]. The significance of hepatotoxic aspects of therapy with erythromycin and related antibiotics has been weighed with a physician's concern[90].

Peptide antibiotics have been reviewed with emphasis on topochemical aspects[91] and structural features[92] particularly the role of dehydroamino acid units[93]. The behavior of α-aminoadipyl- and glutamylcysteine in the presence of intact and disrupted mycelium of a Cephalosporium sp. forms the subject of a detailed analysis[94] of biochemical transformations, involving penicillin N, Cephalosporin C, and others.

Phosphorus-containing antibiotics - The surprising discovery of phosphonomycin by an American-Spanish team[95] revealed a novel structure (XVI), (-)-(1R, 2S)-1, 2-epoxypropylphosphonic acid, which was confirmed by synthesis. Its bactericidal action features a covalent attachment of phosphonomycin to a transferase involved in peptide synthesis of essential cell wall precursors. The antibiotic, in spite of its highly ionic character, is rapidly absorbed from the gut and is effective in man against a variety of infections including those of the urinary tract with E. coli and Alkalingenes fecalis strains as the causative organisms.



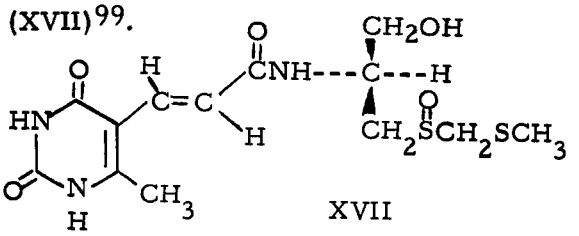
XVI

The results of further clinical evaluation of this antibiotic are awaited with considerable interest.

Diumycin, a new antibiotic with remarkable prophylactic activity[96], and macarbomycin[97], a growth promoting agent in swine, are new members of this class.

The labile chromophore of moenomycin, also present in macarbomycin[97] has been identified as 2-aminocyclopentane-1, 3-dione[98], first example of a naturally occurring aminoreductone.

Sparsomysin, an antibiotic known since 1962, has been shown to have the unique structure (XVII)[99].



XVII

Tetracycline - Doxycycline, one of the "longer-acting" analogs, given as a single oral dose of 100 mg. gives serum levels in man equal to those given by a single oral dose of 250 mg. of tetracycline[100] and appears in inflamed tissue more rapidly, in greater concentration, and persists longer than tetracycline[100]. Adequate serum levels of doxycycline have been attained in patients with renal insufficiency[101]. The relative merits of doxycycline among other tetracyclines have been assessed with reference to its margin of safety in patients with depressed renal function[102].

A perusal of the vast field of antibiotic research elicits a reaction recorded before[103].

References

1. M. Finland, J. Infect. Dis., 120, 620 (1969).
2. R. H. Parker and P. Y. Patterson, J. Chron. Dis., 21, 719 (1969).
3. J. Crofton, Brit. Med. J., a) 2, 137 (1969); b) 2, 209 (1969).
4. S. J. Eykyn and I. Philips, Practitioner, 203, 501 (1969).
5. Antibiotica et Chemotherapia, O. Gsel, Ed., 14, S. Karger, Basel - New York, 1968.
6. Symposium on the Comparative Value of Antibiotics Active Against Gram-Negative Bacilli, Pasteur Institute, Paris, December 5, 1968, Pathologie et Biologie, 17; a) J. Monnier, R. Bourse, N. Moatti and Ch. Suc., 817; b) J. Flourett, S. Chassignol and A. Moulin, 849; c) J. C. Burdin, M. Weber, A. Imbs, M. A. Regnie and J. C. Georges, 855 (1969).
7. International Symposium on Gentamicin, A New Aminoglycoside Antibiotic, University of Illinois, College of Medicine, Chicago, Illinois, October 30-31, 1968; J. Infect. Dis., 119; a) C. E. Cox, p. 486; b) H. H. Stone, p. 504; c) C. M. Martin, A. J. Cuomo, M. J. Geraghty, J. R. Zager and T. C. Mandes, p. 506; d) D. H. Smith, p. 378; e) J. Davies, R.

Benveniste, K. Kvitek, B. Ozanne and T. Yamada, p. 351; f) W. M. M. Kirby and H. C. Standiford, p. 361; g) J. A. Waitz and M. J. Weinstein, p. 355; h) M. Finland, p. 536; j) C. B. Smith, P. E. Dans, J. N. Wilfert and M. Finland, p. 370; k) D. J. Cooper, H. M. Marigliano, M. J. Yudis and T. Traubel, p. 342; m) A. W. Nunnery and H. D. Riley, Jr., p. 460; n) J. O. Klein, p. 457; p) D. B. Louria, L. Young, D. Armstrong and J. K. Smith, p. 483; q) C. E. Cox, p. 486; r) A. W. Nunnery and H. D. Riley, Jr., p. 402; s) J. E. Hawkins, Jr., L-G. Johnsson and J-M. Aran, p. 417; t) J. Wersäll, P. -G. Lundquist and B. Björkroth, p. 410 (1969).

8. G. P. Bodey, V. Rodriguez and J. K. Luce, Amer. J. Med. Sci., 257, 408 (1969).
9. M. E. Levison, W. D. Johnson, T. S. Thornhill and D. Kaye, J. Amer. Med. Assoc., 209, 1331 (1969).
10. G. A. Olsen and G. Lomholt, Brit. J. Vener. Dis., 45, 144 (1969).
11. D. A. Leigh, J. leFranc and A. R. Turnbull, ibid, 151 (1969).
12. J. E. Martin, Jr., A. Lester, D. S. Kellog, Jr. and J. D. Thayer, Appl. Microbiol., 18, 21 (1969).
13. A. Fletcher, P. S. York, E. Musser and R. R. Landes, Clin. Med., 76, 15 (1969).
14. Proceedings of the Scandinavian Symposium on Capreomycin, Ethambutol and Rifampicin, Helsinki, April 25-26,; Scand. J. Resp. Dis., Suppl. No. 69, Munksgaard, Copenhagen, 1969; T. M. Wilson, p. 33.
15. D. H. Smith and P. Gardner, New Eng. J. Med., 282, 161 (1970).
16. R. A. Gleckman and M. A. Madoff, ibid 281, 677 (1969).
17. P. Gardner, D. H. Smith, H. Beer and R. C. Moellering, Jr., Lancet, 2, 774 (1969).
18. C. E. Davis and J. Anandan, New Eng. J. Med., 282, 117 (1970).
19. R. T. Jones and R. Curtiss, Bacteriol. Proc., 66 (1969).
20. H. W. Smith, The Lancet, 1, 1174 (1969).
21. H. Jarolmen and G. Kemp, J. Bact., 99, 487 (1969).
22. H. Jarolmen and G. Kemp, ibid, 97, 962 (1969).
23. T. H. Jukes, Letter to the Editor, New Eng. J. Med., 282, 49 (1970).
24. B. Ozanne, R. Benveniste, D. Tipper and J. Davies, J. Bact., 100, 1144 (1969).
25. J. H. Hartwood and D. H. Smith, ibid, 97, 1262 (1969).
26. R. E. Benveniste, B. W. Ozanne and J. Davies, Bacteriol. Proc., 48 (1969).
27. O. Doi, S. Kondo, N. Tanaka and H. Urezawa, J. Antibiotics, 22, 273 (1969).
28. Abstracts "Ninth Interscience Conference of Antimicrobial Agents and Chemotherapy", American Society for Microbiology, Washington, D. C. October 27-29, 1969: (Papers will appear in "Antimicrobial Agents and Chemotherapy, 1969"); D. H. Smith, J. Janjigian and N. Prescott, p. 55.
29. W. Th. Shier, K. L. Rinehart, Jr. and D. Gottlieb, Proc. Nat. Acad. Sci., 63, 198 (1969).
30. C. M. Kunin, D. Brandt and H. Wood, J. Infect. Dis., 119, 132 (1969).

31. G. L. Hobby, T. F. Lenert and J. Maier-Engallena, Am. Rev. Resp. Dis., 99, 453 (1969).
32. F. Grumbach, Tubercle, 50 (Suppl.), 12 (1969)
33. H. D. Cohn, J. Clin. Pharmacol., 9, 118 (1969).
34. N. Maggi, S. Furesz, R. Pallanza and G. Pelizza, Arzneimittel Forsch., 19, 651 (1969).
35. H. Beeuwkes, H. J. Buytendijk and F. P. V. Maeson, ibid, 1283 (1969).
36. P. W. Brickner, J. Clin. Pharmacol., 9, 243 (1969).
37. W. B. Deal and E. Sanders, New Eng. J. Med., 281, 641 (1969).
38. a) Chr. Virchow and J. Flemming, Beiträge zur Klinik und Erforschung der Tuberkulose und der Lungenkrankheiten, 140, 40 (1969); b) Anon., New Drugs Against Tuberculosis, Lancet I, 1081 (1969); c) E. H. Orlowski, Prax. Pneumol., 23, 178 (1969).
39. R. J. W. Rees, J. M. H. Pearson and M. F. R. Waters, Brit. Med. J., 1, 89 (1970).
40. V. Prelog quoted by M. Staehelin, See Chapter 15, ref. 5, page 60.
41. C. Siddhikol, J. W. Erbstoeszer and B. Weisblum, J. Bact., 99, 151 (1969).
42. F. Knüsel, H. Bickel and W. Kump, Experientia, 25, 1207 (1969).
43. G. C. Lancini and C. Hengeller, J. Antibiotics, 22, 637 (1969).
44. T. Kishi and K. Kamiya, Experientia, 25, 901 (1969).
45. J. A. Ostrenga, J. Med. Chem., 12, 349 (1969).
46. K. E. Price, D. R. Chisholm, F. Leitner, M. Misiek and A. Gourevitch, Appl. Microbiol., 17, 881 (1969).
47. E. Bengtsson, E. B. Holmgren and G. Tunevall, Scand. J. Infect. Dis., 1, 71 (1969).
48. K. Gritz and P. Naumann, Arzneimittel Forsch., 19, 1237 (1969).
49. E. J. L. Lowbury, H. A. Lilly, A. Kidsen, G. A. J. Ayliffe and R. J. Jones, Lancet, 2, 448 (1969).
50. C. H. Schneider and A. L. DeWeck, Int. Arch. Allergy, 36, 129 (1969).
51. M. A. Schwartz, J. Pharm. Sci., 58, 643 (1969).
52. E. S. Wagner, W. W. Davis and M. Gorman, J. Med. Chem., 12, 483 (1969).
53. J. T. Smith, J. M. T. Hamilton-Miller and R. Knox, J. Pharm. Pharmac., 21, 337 (1969).
54. C. W. Ryan, R. L. Simon and E. M. Van Heyningen, J. Med. Chem., 12, 310 (1969).
55. Abstracts, Ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Washington, D. C., October 27-29, 1969; D. R. Chisholm, F. Leitner, M. Misiek and K. E. Price, p. 30.
56. K. Kariyone, H. Harada, M. Kurita and T. Takano, J. Antibiotics, 23, 131 (1970).
57. R. L. Perkins, E. J. Smith and S. Saslaw, Amer. J. Med. Sci., 257, 116 (1969).

58. J. Kump, J. Moskowitz, N. J. Ehrenkranz and Y. Panunzio, Southern Med. J., 62, 461 (1969).
59. H. M. Trafton and H. E. Lind, J. Urology, 101, 392 (1969).
60. R. R. Landes, I. Melnick, A. Fletcher and B. McCormick, ibid, 102, 246 (1969).
61. B. R. Meyers, K. Kaplan and L. Weinstein, Clin. Pharmacol. Ther., 10, 810 (1969).
62. R. J. Fass, R. L. Perkins and S. Saslaw, Amer. J. Med. Sci., 259, 187 (1969); ref. 55, M. Stillerman and H. D. Isenberg, p. 25
63. H. R. Sullivan, R. E. Billings and R. E. McMahon, J. Antibiotics, 22, 195 (1969).
64. M. S. Manhas and A. K. Bose, Synthesis of Penicillin, Cephalosporin C and Analogs, Marcel Dekker, Inc., New York, 1969.
65. J. P. Clayton, J. H. C. Nayler, R. Southgate and E. R. Stove, Chem. Comm., (D3), 129 (1969).
66. O. K. J. Kovacs, B. Ekstrom and B. Sjöberg, Tet. Lett., 23, 1863 (1969).
67. D. A. Johnson, D. Mania, C. A. Panetta and H. H. Silvestri, ibid, 16, 1903 (1968).
68. M. L. Sassiver and R. G. Shepherd, ibid, 45, 3993 (1969).
69. S. Kukolja, R. D. G. Cooper and R. B. Morin, ibid, 39, 3381 (1969).
70. S. Wolfe, R. N. Bassett, S. M. Caldwell and F. I. Watson, J. Amer. Chem. Soc., 91, 7205 (1969).
71. D. H. R. Barton, F. Comer and P. G. Sammes, ibid, 1529 (1969).
72. R. D. G. Cooper, P. V. DeMarco and D. O. Spry, ibid, 1528 (1969).
73. R. D. G. Cooper, P. V. DeMarco, C. F. Murphy and L. A. Spangle, J. Chem. Soc., (C), 340 (1970).
74. R. D. G. Cooper, P. V. DeMarco, J. C. Cheng and N. D. Jones, J. Amer. Chem. Soc., 91, 1408 (1969).
75. R. A. Archer and P. V. DeMarco, ibid, 1530 (1969).
76. R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon and S. L. Andrews, ibid, 1401 (1969).
77. J. A. Webber, E. M. VanHeyningen and R. T. Vasileff, ibid, 5674 (1969).
78. D. Michaeli, B. Meyers and L. Weinstein, J. Infect. Dis., 120, 488 (1969).
79. J. Fedorko, S. Katz and H. Allnoch, Appl. Microbiol., 18, 869 (1969).
80. Ref. 55, K. E. Price, D. R. Chisholm, F. Leitner and M. Misiek, p. 31.
81. F. Daschner and W. Marget, Arzneimittel Forsch., 19, 1003 (1969).
82. B. J. Magerlein and F. Kagan, J. Med. Chem., 12, 974 (1969).
83. A. D. Argoudelis, J. H. Coats, D. J. Mason and O. K. Sebek, J. Antibiotics, 22, 309 (1969).
84. S. Omura, A. Nakagawa, M. Otani, T. Hata, H. Ogura and K. Furshata, J. Amer. Chem. Soc., 91, 3401 (1969).
85. H. Tsukiura, M. Konishi, M. Saka, T. Naito and H. Kawaguchi, J. Antibiotics, 22, 89 (1969).
86. Ref. 55, R. B. Morin, P. V. DeMarco, R. L. Hamill and M. Gorman, p. 17.

87. a) A. K. Mallams, R. S. Jaret and H. Reimann, J. Amer. Chem. Soc., 91, 7506 (1969); b) M. J. Weinstein, G. H. Wagman, J. A. Marquez, B. T. Testa, E. Oden and J. A. Waitz, J. Antibiotics, 22, 253 (1969).
88. L. A. Mitscher, B. J. Slater, T. J. Perun, P. H. Jones and J. R. Martin, Tet. Lett., 52, 4505 (1969).
89. P. V. DeMarco, J. Antibiotics, 22, 327 (1969).
90. P. Braun, J. Infect. Dis., 119, 300 (1969).
91. M. M. Shemyakin, Y. A. Ovchinnikov and V. T. Ivanov, Angew. Chem. Internat. Ed., 8, 492 (1969).
92. M. Bodanszky and D. Perlman, Science, 163, 352 (1969).
93. B. W. Bycroft, Nature, 224, 595 (1969).
94. P. Bronwen Loder, E. P. Abraham and G. G. F. Newton, Biochem. J., 112, 389 (1969).
95. B. G. Christensen, W. J. Leanza, T. R. Beattie, A. A. Patchett, B. H. Arison, R. E. Ormond, F. A. Kuehl, Jr., G. Albers-Schonberg and O. Jardetzky, Science, 166, 123 (1969).
96. E. Meyers, D. S. Slusarchyk, J. L. Bouchard and F. L. Weisenborn, J. Antibiotics, 22, 490 (1969).
97. S. Takahashi, A. Okanishi, R. Utahara, K. Nitta, K. Maeda and H. Umezawa, ibid, 23, 48 (1970).
98. R. Tschesche, D. Lenoir and H. L. Weidenmuller, Tet. Lett., 3, 141 (1969).
99. P. F. Wiley and F. A. MacKellar, J. Amer. Chem. Soc., 92, 417 (1970).
100. G. M. Williamson, Chimica Therapeutica, 4, 53 (1969).
101. W. Stein, M. Schoog and H. E. Franz, Arzneimittel Forsch., 19, 827 (1969).
102. Anon., Evaluation of a New Antibacterial Agent, J. Amer. Med. Assoc., 209, 549 (1969).
103. Gospel according to St. John, Chapter 21, verse 25b.

Chapter 10. Synthetic Antibacterial Agents

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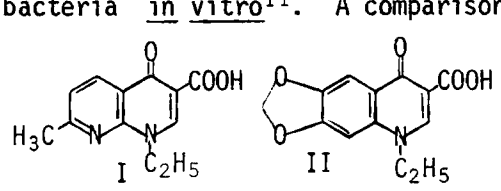
During 1969, no novel chemical classes of antibacterial agents with clinical effectiveness were reported. Synthetic efforts dealt mainly with the modification of existing drugs with the development of several promising leads. A multiple-drug therapeutic regimen has been advocated for the treatment of urinary tract infections[1], chronic bronchitis[2] and tuberculosis[3,4]. Wide-spread use of the multiple-drug technique may be the answer to the problems of development of resistant strains and R-factor transmission that beset the present day chemotherapeutic approach to disease.

The organization of this review is similar to that used in previous years with the exception that compounds having only in vitro activity are discussed with the corresponding class of closely related systemic agents, such as nitrofurans, sulfonamides and antituberculous drugs. This arrangement should allow for an insight into structure-activity relationships.

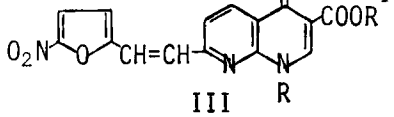
Quinolone Antibacterial Agents

Nalidixic acid I inhibits DNA synthesis in both infected and uninfected cells but has little effect upon bacteriophage DNA synthesis[5] and may be useful for studies of virus specific DNA synthesis. Hemolysin production has been implicated[6] in resistance by E.coli to I and the major effect of microbial degradation was found[7] to be hydroxylation. A review of the clinical use of I attested to its usefulness for the treatment of urinary tract infections[8]. Nalidixic acid proved the most effective of 8 clinically used agents against ca. 3000 clinical isolates from urinary tract infections[9]. The bacteriostatic activity of I against aerobic gram-negative bacterial strains[10] was partially reduced by nitrofurantoin and a 1:1 mixture of I and neomycin was synergystic against a broad spectrum of bacteria in vitro[11]. A comparison of in vitro and in vivo activities



I

II



III

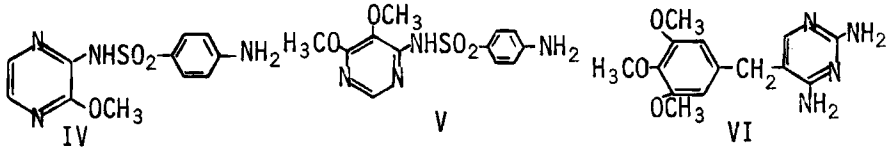
showed oxolinic acid II to be at least 10-fold more active in vitro against enterobacteriacae than I with neither very effective against Pseudomonas strains. Clinical trial in 54 patients indicated that oxolinic acid II (2g/day) was associated with less emergence of resistance and had a distinct potential advantage over nalidixic acid I (4g/day)[12]. Several papers concerning the treatment of urinary tract infections with oxolinic acid II were presented[13,14] with results comparing favorably to those obtained with I.

Derivatives - Styryl derivatives III of nalidixic acid were prepared and

reported[15,16] to exhibit potent in vitro activity against various unspecified gram-negative and gram-positive organisms. This group includes compounds in which R=alkyl or substituted alkyl and R'=H, alkyl, substituted alkyl and a variety of amide derivatives. Hydration of the vinyl group or absence of the nitro group markedly decreased activity and aminoalkyl esters were more active than alkyl esters or amides. Detailed microbiological results are pending.

Sulfonamides

Clinical evaluation of the newer long-acting sulfonamides yielded generally favorable results. Sulfasymazine(2-sulfanilamido-4,6-diethyl-1,3,5-triazine) gave up to 75% cures in recurrent urinary tract infections[17]. Sulfalene IV showed excellent results in pediatric practice with conditions treated including cystitis, bronchitis, pneumonia and purulent otitis[18]. The use of IV in the treatment of urinary tract infections[19], rheumatic disease[20], and some pharmaco-kinetic aspects were discussed[21]. Sulformethoxine V has been compared to other commercially available sulfonamides[22]. It is distinguished by very slow elimination from plasma due probably to a high rate of tubular reabsorbtion. Since the half-life averages 179 hr., effective therapeutic levels can be maintained by weekly doses[22,23]. It has a particularily broad spectrum spanning bacteria, fungi and plasmodia. An established drug, sulfisoxazole has proven superior to tetracycline for the treatment of chancroid[24], a frequent venereal disease among military personnel in Vietnam. The drug is also of value in the treatment of subclinical renal infections during pregnancy[1]. Mitchell[25] in a review on sulfonamides for children, considers IV and other long-acting sulfonamides contraindicated due to the occurrence of the Stevens-Johnson syndrome.

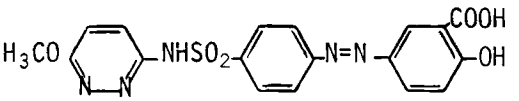


IV V VI

The synergy of sulfonamides and a folate metabolism inhibitor, trimethoprim VI, received extensive laboratory and clinical evaluation[26]. The combination of sulfamethoxazole and VI was frequently found superior to ampicillin or the sulfonamide alone[26-29]. The combination has been successfully used in the treatment of uro-genital[26,30,31], renal[26,32] and respiratory infections[26,33]. Favorable results were also obtained in cases of typhoid fever, S. typhi carriage, fulminating Staph. infections[26] and prevention of infection due to drug induced bone marrow depression[34]. Reports linking undesirable hematological effects (agranulocytosis and thrombocytopenia) to administration of sulfamethoxazole and VI have appeared[35,36].

In vivo studies showed IV to be inferior to sulfamethoxypyridazine in protecting mice against S. aureus or K. pneumonia[37]. Higher therapeutic activity (in induced septicemia in mice), lower toxicity and more rapid absorption may make salicylazosulfamethoxypyridazine VII[38] more important clinically than salicylazosulfapyridine. The latter has been effective in

the treatment of non-specific ulcerative colitis[39,40]. Species difference in the metabolism and excretion of sulfonamides have been studied by several groups[41-43].



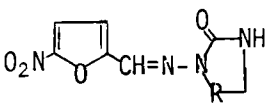
VII

Synthetic activity in this field involved the preparation of sulfanilamide derivatives of 1,4-naphthoquinones[44], benzisothiazol-1,1-dioxide[45], 5-methylthiopyrimidines[45], 6-aminopenicillanic acid[46], adamantamine[47] and succinanilic acid[48]. In vitro test data were reported for the latter three groups with the adamantane derivatives being inactive. Sulfonamides of carbazolesulfonic acid[49], α- and β-naphthylaminosulfonic acids[50], and p-alkoxybenzenesulfonic acids[51] were prepared. All had some activity in vitro, with the biguanides of the latter group active against M. smegmatis[51]. The introduction of the nitrofurfurylidene group into a sulfanilamide resulted in an increase (up to 62.5 fold) in in vitro activity[52].

Theoretical Aspects - A linear relationship was observed between bacteriostatic activity and modified Hammett substituent parameters for a series of meta and para substituted N_1-phenylsulfanilamides[53]. Enzyme-substrate complexes may be charge-transfer complexes formed in the living organism and sulfonamides are believed to interfere with the utilization of PABA through competitive enzyme inhibition. An investigation of these postulates[54] disclosed a relationship between the charge-transfer transition energy or ionization potential of sulfonamides and their in vitro bacteriostatic activity.

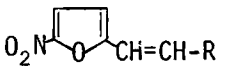
Nitrofuran and Related Antibacterials

Several clinical studies comparing nitrofurantoin VIII to other drugs in the treatment of urinary tract infections, indicated VIII was more efficient against E.coli infections than streptomycin or sulfamethazine[55] and had a higher 2-year post-treatment cure rate against a spectrum of organisms than cycloserine or sulfamethazine[56]. The site of action of VIII was investigated[57] with buildup found in the kidneys of rats. Although blood levels were low, levels in kidney tissue were greater than MIC values for

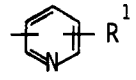


VIII R=C(=O)NH

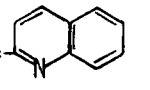
IX R=CH_2



X R=



XI R=

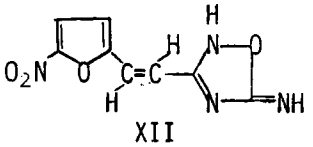


common urinary tract pathogens. Intracellular accumulation of VIII was studied[58] in rabbit renal cortical slices and the metabolism, with different dosage forms and by different routes, was studied in dogs[59]. In one study of 81 patients with urinary tract infections[60], nifuradene IX was

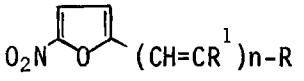
found equivalent to VIII. In another study[61], a 70% cure rate was obtained with IX on an 800mg/kg, 14 day regimen.

A comparison between the activity of nitrofuryl vinyl heterocyclics of types X and XI and their corresponding N-oxides was undertaken[62]. The N-oxides were found considerably more active in vitro and in vivo against S.aureus and Salmonella typhimurium in mice, with higher antibacterial serum levels. Russian workers[63] have also prepared XI and found it to have high in vitro activity against Proteus species and Pseudo. aeruginosa. It was found that XI was inactivated by both horse and human serum while seven clinically useful nitrofurans were not significantly affected by sera and were effective in combating gram-negative induced septicemia in mice. A series of 3- and 5-amino-1,2,4-oxadiazoles were investigated[64] and found to have broad spectrum antibacterial properties. The trans tautomer XII was reported[65] to be highly active and unaffected by serum or pH variations in the range 5-8. Cross resistance developed with nitrofuran type antibacterials but not with other agents. Metabolism is rapid and complete in mice and rats. The oral LD_{50} in mice is 6g/kg and the in vivo spectrum is comparable to furazolidone. A group of 55 3- and 5-nitrofuryl isoxazoles[66] were found to induce phage production in lysogenic bacteria, but were relatively inactive in vivo.

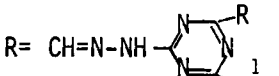
A series of hydrazino-1,3,5-triazines XIII were found[67] to have good in vitro and moderate in vivo activity against M.tuberculosis. The most



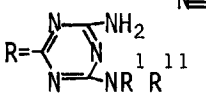
XII



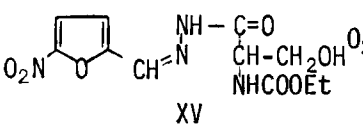
XIII



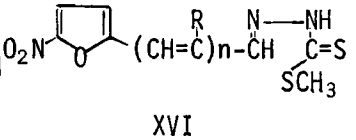
XIV



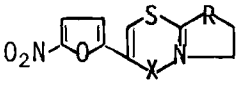
active (n=0 and R=R'=isopropylamino) had an MIC of 1μg/ml against an INH resistant strain. A group of 26 related triazino compounds of type XIV were found[68] to have high activity against both gram-negative and gram-positive organisms. Acetylation did not significantly alter potency but when n=0, activity was markedly decreased. Nitrofuryl-1,2,4-triazoles were compared to nitrofurantoin VIII and some found[69] to be equally effective in the treatment of experimentally induced E.coli pyelonephritis in rats. The most active compound in a series of nitrofurfurylidene-aminohydantoins and N-(ethoxycarbonyl) amino acid hydrazides was XV. The compounds were active in vitro and in vivo, with XV having an ED_{50} of 96mg/kg against S.typhosa, p.o. in mice[70].



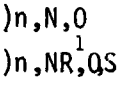
XV



XVI



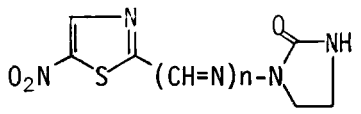
XVII



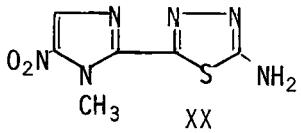
In a group of methyldithiocarbazonates of type XVI, activity against both gram-negative and gram-positive organisms was found[71]. When R was other

than H, the activity and toxicity were markedly decreased. Nitrofuryl cinchoninic acid derivatives[72] were investigated and the most active compound had an ED_{50} (in mice) of 5mg/kg, i.p. against S.aureus, but was inactive orally. Some analogs and derivatives of furazolium XVII (X=$(CH_2)_0$ R=NH) were investigated[73] with none of the compounds exhibiting the same order of activity as furazolium. 2-(5-NO_2furyl)vinyl-Quinoxaline derivatives were reported[74] to have moderate in vitro activity against the usual organisms but relatively high activity against M.tuberculosis. Nitrofurylpyranobenzoxazoles were active against M.tuberculosis[75], with the most active having an MIC of 1.6µg/ml. N-Aryl and N-heterocyclically substituted nitrofuryl nitrones had, in contrast to the non-nitrone analogs, only slight in vitro and essentially no in vivo activity[76]. The replacement of the nitro group by the methanesulfonyl group in a variety of furyl amides, thiosemicarbazides, etc. virtually eliminated all in vitro activity[77].

1-hydroxyalkyl 2- or 5-nitroimidazoles[78] had both in vitro and in vivo activity, inhibiting anaerobic but not aerobic bacteria. Nitrothiazoles were investigated[79] and the only in vivo active compound in a series of 30 was XVIII, having about 50% of the activity of furazolidone against S. aureus in mice. Niridazole XIX, the most active of 18 related compounds was found[80,81] to have from 1/3 to 2X the oral effectiveness of chlor-



XVIII n=1
XIX n=o



XX

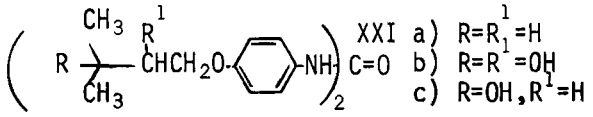
amphenicol against a panel of organisms. Results in calves suggested its use in the treatment of Salmonellosis or Shigellosis. The in vitro and in vivo activities of the thiadiazole XX, announced last year, were reported in depth[82,83]. Against experimental Salmonella infections in swine it proved more effective than furazolidone[82] and in mice and in vitro it was equipotent with nalidixic acid I against gram-negative organisms. It was active against gram-positive organisms (with I essentially inactive) but less than that of clinically useful drugs[83].

Antitubercular Agents

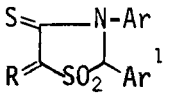
No real breakthrough in tuberculosis chemotherapy has occurred this year and although a number of in vivo active drugs were prepared and studied, they were generally less effective than drugs presently in clinical use.

A six year clinical study with isoniazid (INH) therapy was reported[84] and a review and evaluation of the clinical use of ethambutol against tuberculosis appeared[85].

The ingestion of thiocarlide XXIa by humans was found[86] to give rise to two metabolites XXIb and XXIc. Metabolite XXIb was equal to XXIa in



XXI a) $R=R_1=H$
b) $R=R^1=OH$
c) $R=OH, R^1=H$



XXII

activity while XXIc had only ca. 10% of the activity of the parent compound. Substitution of one of the para alkoxy chains of XXIa by aryl, styryl, heterocyclic or aryloxy groups resulted in a group of 283 compounds, many with good in vitro activity[87]. Some of the more active compounds exhibited fair in vivo activity (p.o. in mice) but were not as good as drugs in clinical use. The replacement of one of the phenyl groups in XXIa by a benzyloxy group led to a number of in vitro active compounds[88]. The most active exhibited in vivo activity in mice only at or near the toxic dose. A series of compounds exemplified by XXII were prepared and several were found active at $1:10^6$ dilution in vitro against M.tuberculosis[89]. These compounds were reported to be active in animals and man but were not better than presently used drugs.

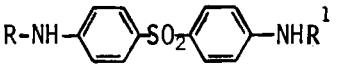
The major problem in tuberculosis therapy is the buildup of resistance to the drug in use. Attempts at overcoming this difficulty took the form of additives such as substituted phenothiazines and acridines to retard emergence of resistance to INH[90,91] or combinations of drugs such as INH plus ethionamide, pyrazinamide, cycloserine or ethoxide[92]. The biophysical aspects of the interaction of amino acridines with nucleic acids were reviewed[93].

In Vitro Active Antitubercular Agents

A considerable number of papers appeared concerning the preparation and testing of a variety of drugs active in vitro against M.tuberculosis. These include: substituted benzylidene isonicotinic hydrazides[94,95], 4-aryl-1-aryloxybenzyl semicarbazones[94], (phenylthio)acetohydroxamic acids[96], bis-(phenylenedioxy)acetamides acethydrazides and acetohydroxamic acids[96], hydroxyphenyl acethydrazides[96], pseudothiohydantoins[94], ethyl α-cyano cinnamate derivatives[97], aminoalkylamino phenyleneazo-bis-sulfones[98], p-thiazolyl thiocarbanilides[99], pyridazo thiocarbanilides[100], phenylhydrazides of diaryl and dialkyl glycolic acids[101], 2-(2-benzothiazolylthio) and 2-(benzoxazolylthio) thioacetamides[102], 1-phenyl-3-substituted benzothiazolyl guanidine derivatives[103,104], 3-substituted-7-amino-4-hydroxycoumarins[105], and basic derivatives of ring A-nor androstanes[106].

Leprosy

The syntheses and some pharmacological properties of mono- (XXIIIb) and diacetyldapsone (XXIIIc) were reported[107]. Investigation of repository dapsone polymers resulted in the preparation of XXIV , which was found to have a more intense but less prolonged effect than XXIIIb and to be safer than dapsone XXIIIa. This was manifested by the appearance of only a low order of methemoglobin, a major problem with prolonged dapsone therapy.

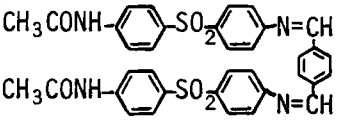


XXIII

XXIII a) $R=R^1=H$ dapsone

b) $R=COCH_3$ $R^1=H$

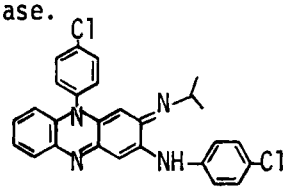
c) $R=R^1=COCH_3$



XXIV

PSBA XXIV was reported[107] to have an oral LD_{50} of 3g/kg in rats and effective repository action against M.tuberculosis. Similar polymers were also found active in mice against M.leprae[108], and completely suppressed the disease when administered at 200mg/kg, s.c. at 8 week intervals, after an initial 400mg/kg dose.

A group of compounds active against mycobacteria were studied against the apparently unique phenoloxidase found in M.leprae[109]. The most active inhibitor of the enzyme was found to be diethyl dithiocarbamate with XXIIIa having about ½ this activity. Since this enzyme was not found elsewhere, a non-toxic enzyme inhibitor was considered a possible approach to a rational chemotherapy of the disease.

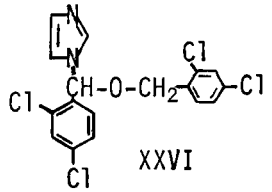


XXV

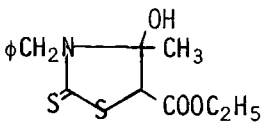
A study of clofazimine XXV against M.leprae in mice[110], revealed that it had 100 times the activity of ethionamide and was equipotent with XXIIIa when administered in the diet. Buildup in the tissues accounted for the long post-administration effectiveness of XXV. This compound was found to be both bacteriostatic as well as bacteriocidal and synergistic with INH against M.leprae[111].

Miscellaneous In Vivo Active Compounds

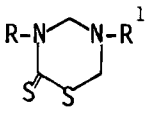
A group of 1-substituted imidazoles were found active against gram-positive organisms with the most active XXVI totally inhibiting E.insidiosa and S.hemolyticus at 0.01μg/ml. This compound is presently undergoing clinical evaluation[112]. A series of 228 heterocyclic compounds which are



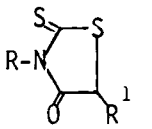
XXVI



XXVII



XXVIII



XXIX

potential "isothiocyanate formers" was prepared[113] and found to have in vitro activity against a broad spectrum of bacteria and fungi. The most active compound was XXVII, with examples of types XXVIII and XXIX also active. Another paper[114] dealt with 104 compounds of type XXVIII, wherein R= alkyl or aralkyl and R'=groups with hydrophilic substituents for best

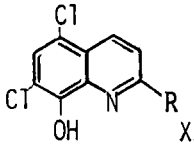
activity. Oral administration to dogs and guinea pigs led to bactericidal urines.

Cetophenicol, a synthetic analog of chloramphenicol having an acetyl group in place of the nitro group, is immunologically different from chloramphenicol. In infected animals, cetophenicol stimulates the proliferation of antibody-producing spleen cells against foreign erythrocytes and bacteria while chloramphenicol inhibits this response[115,116].

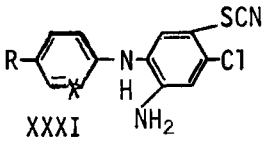
Antiseptics, Topical Agents and Soap Bacteriostats

In a serialized article, Gucklhorn[117] thoroughly reviewed the use of antimicrobials in cosmetics. A review of agents used in ophthalmic practice[118] includes disinfectants and antibacterials and indications for their use.

Simple α- and β-glycols, glycol ethers[119] and dimethyl sulfoxide (DMSO)[120] were examined as solvents for antimicrobial testing. In general, >3% of a glycol or glycol ether and >15% of DMSO inhibited a broad spectrum of test organism. The antimicrobial action of soap germicidal mixtures were studied against test organisms[121] including S.aureus, E.coli and A. niger. Hexachlorophene, 3,4,4'-trichlorocarbanilide (TCC) and 3,4',5-tribromosalicylanilide (TBS) were equipotent against S.aureus but TBS was most effective against E.coli and A.niger. The study of the mechanism of action of salicylanilides[122] implicated impairment of cellular respiration. The localization of germicides in skin was investigated[123] using TCC and zinc or zirconium chelate salts of 2-pyridinethiol-1-oxide (pyrithiones). Nine pyrithiones were found[124] to be highly active against a broad spectrum of organisms with several equal in potency to cetol (cetyldimethylbenzylammonium chloride). 1-Dichlorophenyl-3-acyl ureas were evaluated as soap bacteriostats[125] with MIC values of 0.1-0.5μg/ml against S.aureus. A group of substituted phenyl azo phenols were found to have ca. 100x the potency of chloramphenicol[126]. Twenty-seven azo derivatives of salicylic and α-hydroxynaphthoic acids were tested and several found to be active against penicillin-resistant strains of S.aureus[127]. The study of the action of 5-hexylsalicylidene-p-aminophenol on bacteria[128] indicated inhibition of RNA synthesis, suppression of respiration and aerobic glycolysis, probably by a chelation effect. A series of 17-dihalo-8-hydroxyquinolines was prepared[129] and the most active XXXa was found to have broader bacteriostatic activity than chloroquinaldol. Polynitro-diphenylamines with halogen or



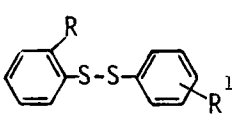
XXX Chloroquinaldol(R=CH_3)
a) R=$NHCOCH_3$



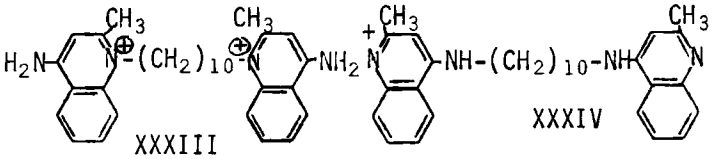
XXXI

trifluoromethyl[130] substituents had high bacteriostatic activity with MIC values of 0.15-1.2μg/ml against gram-positive bacteria. Diarylamines XXXI (wherein R=halo or alkoxy and X=N or C) were reported[126] to be effective antiseptics with at least 100x the *in vitro* potency of chloramphenicol. A

phenyl disulfide of type XXXII (with R=R'=CONHϕ) was found to be relatively non-toxic and had broad-spectrum activity against Micrococcus, Staphylococcus and Bacillus species[131]. In the group wherein R=CN and R'=halo,



XXXII



XXXIII XXXIV

nitro, amino, etc. effective bacteriostats[132] were found.

Utilizing E.coli and S.aureus, the mode of action of dequalinium acetate XXXIII was investigated[133] and the compound was found to be absorbed into the cell, precipitating cytoplasmic materials. The preparation, pharmacology and successful clinical testing of the isomeric compound XXXIV as a 1% topical ointment was reported[134]. Analogs of XXXIV in which the quinaldine moiety is replaced by the 4-pyridyl and 9-acridinyl groups[135] or by 5,6,7,8-tetrahydroquinaldine[136] exhibited somewhat less activity. fluorinated 4-arylamino quinaldines[137] exhibited marked in vitro activity against a wide range of gram-positive organisms.

Classes of metal organic compounds having potent in vitro activity include triphenyl tin esters[138], substituted 1,10-phenanthroline[139,140] and 2,2'-bipyridine[140] metal chelates, trans-dihalotetrapyridine rhodium salts[141], trialkyl stannanol and esters[142] and triphenyl lead isocarboxylates[143].

Miscellaneous In Vitro Active Compounds

The following types of compounds exhibited in vitro activity but were either inactive in vivo or no in vivo data were supplied.

17-β-Amino androstenes[144], amine salts of long-chain carboxylic acids containing ether linkages[145], ampholytes of type $CH_3(CH_2)_nNH(CH_2)_mCOOH$[146], α-amino-β-aroylpropionic acids[147], disubstituted amides of long chain fatty acids and decanediamides[148], hydrazides of naturally occurring amino acids[149], synthetic dipeptides containing cyclopentane and β-2-thienyl groups[150], amides of N-acylcystines and cysteines[151], 1,4-phthalazinediyldihydrazones[152], nitrophenyl esters of cyclophosphinic acids[153], 1,6-naphthyridine-N-oxides and 2-hydroxy derivatives[154], quinone derivatives [benzo(g)quinoxalines and naphtho(2,3-b)1,4-oxazines][155], amino-1,4-naphthoquinone imines[156], α-aryl-β-amino aryl ketones[157], Mannich bases of 3-hydroxycoumarin[158], aminoalkyl esters benzyl dithiocarbamates[159], dialkylaminoalkyl biphenyl amines and N-biphenyl piperazines[160], disubstituted hydrazinoalkylamino acridines[161], amino alkanes and derivatives[162], 3-(diphenylmethoxy)-8-isopropylnortropane methanesulfonate[163], imidazothiazoles and thiazolo-pyrimidines[164], 2-aryl-halo substituted pyridazinones[165], diazoimidazole carboxamides and triazeno imidazoles[166], and 3,5-dioxopyrazolidine-4-dithiocarboxylic acid hydrazides[167].

References

1. B.G.Wren, Med. J. Aust., 2, 895 (1969).
2. D.T.D.Hughes, Brit. Med. J., 4, 470 (1969).
3. A.J.Proust, Med. J. Aust., 2, 1052 (1969).
4. J.Feldman, Geriatrics, 24, (11) 92 (1969).
5. L.P.Gage and D.J.Fujita, J. Bacteriol., 98, 96 (1969).
6. J.R.Walton and D.H.Smith, Antimicrobial Agents and Chemotherapy-1968-p. 54.
7. A.Capek, A.Simek, J.Turinova and J.Tuma, Folia Microbiol. (Prague), 14, 94 and 557 (1969).
8. T.A.Stamey, N.J.Nemoy and M.Higgins, Investigative Urology, 6, 582 (1969).
9. R.Chamfeuil and H.Curcier, Presse Med., 77, 1763 (1969).
10. J.D.Piguet, M.Rossi and J.Schmid, Ann. Inst. Pasteur, Paris, 116, 19 (1969).
11. R.Auriti and L.Ravagnan, Antibiotica, 6, 72 (1969).
12. E.Atlas, H.Clark, F.Silverblatt and M.Turck, Ann. Intern. Med., 70, 713 (1969).
13. J.H.Peters, paper presented before Int. Cong. of Chemotherapy, Tokyo, Aug. 1969.
14. J.P.Truant and C.Katsampas, ibid.
15. S.Nishigaki, F.Yoneda, K.Ogiwara, T.Naito and R.Dohmori, Chem. Pharm. Bull., 17, 1827 (1969).
16. R.Dohmori, S.Kadoya, Y.Tanaka, I.Takamura, R.Yoshimura and T.Naito, ibid.
17. R.S.Nanra, M.Bailie and P.Kincaid Smith, Med. J. Aust., 2, 1258 (1969).
18. D.Baetgen, Med. Mschr., 23, 368 (1969).
19. W.Straube, Med. Welt., 1969, 1093.
20. E.Mayr, Pharm. Praxis, 1969, 234.
21. H.Held, L.von Berger and H.F.von Oldershausen, Arzneim.-Forsch., 19, 98 (1969).
22. E.Boeni, B.Fust, J.Rieder, K.Schaerer and L.Havas, Chemotherapy, 14, 195 (1969).
23. Editorial, Ars Medici, 24, 173 (1969).
24. R.E.Kerber, C.E.Rowe and K.R.Gilbert, Arch. Dermatol., 100, 604 (1969).
25. R.G.Mitchell, Practitioner, 204, 20 (1970).
26. Proc. of Conf. at Royal College of Physicians, May 1969, "The Synergy of Trimethoprim and Sulfonamides", Postgrad. Med. J., suppl. Vol. 45 (1969).
27. D.S.Reeves, M.C.Faiers, R.E.Pursell and W.Brumfitt, Brit. Med. J., 1, 541 (1969).
28. R.N.Gruneberg and R.Kolbe, ibid, p. 545.
29. R.N.Morzaria, I.G.Walton and D.Pickering, ibid, 2, p. 511.
30. Special Correspondent, ibid, 2, 507.
31. D.J.M.Wright and A.Grimble, Brit. Med. J., 1, 637 (1969).
32. R.R.Bailey, P.J.Little and G.L.Rolleston, ibid, 1, 550 (1969).
33. Editorial, Practitioner, 203, 817 (1969).
34. R.N.Gruneberg and D.E.Theile, Brit. Med. J., 2, 514 (1969).

35. D.I.K.Evans and R.Tell, ibid, 1, 578 (1969).
36. P.Mohan, Practitioner, 202, 553 (1969).
37. O. Kitamoto and K.Fukaya, Nippon Kagaku Ryohogakukai Zasshi, 16, 765 (1968).
38. E.N.Padeiskaya, L.M.Polukhina and G.N.Pershin, Farmakol. Toksikol. (Moscow), 32, 467 (1969).
39. C.Nicolaidis, E.Kalliterakis, N.Kralios and C.Antoniou, Proc. R. Soc. Med., 62, 262 (1969).
40. M.K.Levitan, Klinich. Med., 47, 102 (1969).
41. S.N.Cohen and W.W.Weber, Biochem. J., 111, 249 (1969).
42. J.W.Bridges, M.R.Kibby, S.R.Walker and R.T.Williams, ibid, p. 167, 173 (1969).
43. T.Arita, R.Hori, E.Owada and K.Takahashi, Chem. Pharm. Bull., 17, 2526 (1969).
44. F.I.Carrol, K.H.Dudley and H.W.Miller, J. Med. Chem., 12, 187 (1969).
45. H.Egg, Mh. Chem., 100, 34, 518 (1969).
46. G.P.Bodey and D.Stewart, Appl. Microbiol., 18, 76 (1969).
47. A.I.Ivanov, M.I.Shmarjan and A.P.Skoldinov, Khim. Farm. Zh., 3, (3) 13 (1969).
48. C.Rodriguez-Salazar, J. Med. Chem., 12, 557 (1969).
49. T.I.Proshechkina, T.I.Shiskina, T.V.Mikhailova and V.V.Kazakova, Khim. Farm. Zh., 3, (5) 9 (1969).
50. R.D.Desai, G.S.Saharia and H.S.Sodhi, J. Indian Chem. Soc., 46, 115, 411 (1969).
51. A.Colautti, M.DeNardo and F.Rubessa, Farmaco, Ed. Sci., 24, 435 (1969).
52. S.Wada, K.Kumaki and I.Moriguchi, Chem. Pharm. Bull., 17, 2168, 2364 (1969).
53. E.R.Garrett, J.B.Mielck, J.K.Seydel and H.J.Kessler, J. Med. Chem., 12, 740 (1969).
54. I.Moriguchi and N.Kaneniwa, Chem. Pharm. Bull., 17, 2554 (1969).
55. A.C.Kennedy, M.E.M.Allison, J.D.Briggs and J.McGeachie, Scot. Med. J., 14, 71 (1969).
56. J.S.Milne, A.W.Irons, G.Kennedy and E.T.Wallace, ibid, p. 76.
57. H.Rocha, E.da Silva Teles and M.Barros, Appl. Microbiol., 18, 547 (1969).
58. J.Andersen, F.Kopko, E.G.Nohle and A.J.Siedler, Amer. J. Physiol., 217, 1435 (1969).
59. J.D.Conklin, R.J.Sobers and D.L.Wagner, J. Pharm. Sci., 58, 1365 (1969).
60. H.K.A.Schirmer and H.Schwarz, J. Urology, 101, 109 (1969).
61. J.M.Malin, Jr., E.E.Anderson and J.F.Glenn, ibid, 102, 345 (1969).
62. M.Shimuzu, S.Nakamura and Y.Takase, Chem. Pharm. Bull., 17, 2330 (1969).
63. L.N.Alekseeva and B.A.Brizga, Latv. PSR Zinat. Akad. Vestis, 7, 102 (1969).
64. H.Breuer, J. Med. Chem., 12, 708 (1969).
65. I.Weliky and E.C.Schreiber, Pharmacologist, 11, 280 (1969); and a series of papers presented at the 9th Interscience Conf. on Anti-Microbial Agents and Chemotherapy (Oct. 1969).
66. R.G.Micetich, J. Med. Chem., 12, 611 (1969).

67. H.Bruhin, X.Buehlmann, W.H.Hook, W.Hoyle, B.Orford and W.Vischer, J. Pharm. Pharmacol., 21, 423 (1969).
68. S.Nishigaki, F.Yoneda, H.Matsumoto and K.Morinaga, J. Med. Chem., 12, 39 (1969).
69. V.Prat, L.Konickova and A.Hammarstroem, Acta Pharmacol. Toxicol., 27, 445 (1969).
70. G.C.Wright, J.G.Michels and C.F.Spencer, J. Med. Chem., 12, 379 (1969).
71. R.Kalnsbergs, S.Hillers, M.Lidaks, L.N.Alekseeva, L.Kruzmetra, B.A. Brizga, A.Y.Zile and I.Petersone, Khim. Farm. Zh., 2, (11) 11 (1968).
72. H.A.Burch, J. Med. Chem., 12, 535 (1969).
73. H.R.Snyder, Jr., and L.E.Benzamin, J. Med. Chem., 13, 164 (1970).
74. N.O.Saldabol, L.N.Alekseeva, B.A.Brizga, K.K.Medne, L.V.Kruzmetra and A.Y.Zile, Khim. Farm. Zh., 3, (3) 9 (1969) and 3, (9) 16 (1969).
75. M.Ichikawa and H.Ichibagase, Chem. Pharm. Bull., 16, 2093 (1968).
76. H.K.Kim and R.E.Bambury, J. Med. Chem., 12, 719 (1969).
77. S.Toyoshima, K.Shimada, K.Kawabe and T.Kanazawa, Yakugaku Zasshi, 89, 779 (1969).
78. H.N.Prince, E.Grunberg, E.Titsworth and W.F.DeLorenzo, Appl. Microbiol., 18, 728 (1969).
79. G.Asato, G.Berkelhammer and E.L.Moon, J. Med. Chem., 12, 374 (1969).
80. F.Kradolfer, R.Jarumilinta and W.Sackmann, Ann. N.Y. Acad. Sci., 160, 740 (1969).
81. P.Schmidt, K.Eichenberger, A.O.Ilvespaa and M.Wilhelm, ibid, p. 530.
82. H.W.Layton, H.J.Bachmann, G.A.Kemp, G.O.Gale and J.S.Kiser, Antimicrobial Agents and Chemotherapy-1968-p. 528.
83. G.S.Redin, N.A.Kuck and M.Forbes, ibid, p. 534.
84. S.Grzybowski, M.J.Ashley and N.E.McKinnon, Can. Med. Ass. J., 101, 81 (1969).
85. Council on Drugs, J. Amer. Med. Ass., 208, 2463 (1969).
86. R.W.Balsiger, T.Leuenberger, W.Michaelis and O.Schindler, Helv. Chim. Acta, 52, 1323 (1969).
87. E.Winkelman, W.H.Wagner and H.Hilmer, Arznein.-Forsch., 19, 543 and 719 (1969).
88. Y.V.Markova, O.O.Makeeva, N.G.Ostroumova, V.I.Lebedeva, M.N.Shchukina and G.N.Pershin, Khim. Farm. Zh., 3, (1) 13 (1969).
89. B.E.Zhitarov and S.N.Baranov, Khim. Farm. Zh., 3, (11) 10 (1969).
90. R.E.Manion, S.G.Bradley and W.H.Hall, Proc. Soc. Exp. Biol. Med., 130, 206 (1969).
91. V.S.Zueva and V.N.Soloviev, Antibiotiki (Moscow), 14, 355 (1969).
92. O.O.Makeeva, Farmakol. Toksikol. (Moscow), 32, 202 (1969).
93. A.R.Peacocke, Chem. Ind. (London), 1969, 642.
94. F.Fujikawa, K.Hirai, S.Konishi, F.Kondo, F.Ueda, M.Hayata, Y.Inone, M.Naito, S.Tsukuma and N.Mabuchi, Yakugaku Zasshi, 89, 1099, 1119, 1266 (1969).
95. F.Knotz, Sci. Pharm., 37, 35 (1969).
96. J.Lange and T.Urbanski, Diss. Pharm. Pharmacol., 20, 589, 599, 607, (1968).
97. C.Luu Duc, J.Alary and A.Noucherle, Chim. Ther., 4, 271 (1969).
98. E.F.Elslager and A.A.Phillips, J. Med. Chem., 12, 519 (1969).

99. B.S.Kulkarni, B.S.Fernandez, M.R.Patel, R.A.Bellare and C.V.Deliwala, J. Pharm. Sci., 58, 852 (1969).
100. N.B.Galstukhova, G.S.Predvoditeleva, I.M.Berzina, T.V.Kartseva, T.N. Zykova, M.N.Shchukina and G.N.Pershin, Khim. Farm. Zh., 3, (4) 7 (1969).
101. I.S.Berdinskii, P.A.Petyunin, O.O.Makeeva, T.M.Dimitrieva and G.N. Pershin, ibid, p. 15.
102. T.P.Sycheva, T.N.Pavlova, T.N.Zykova and M.N.Shchukina, ibid, 3, (8) 18 (1969).
103. P.N.Bhargava and H.Singh, J. Med. Chem., 12, 558 (1969).
104. M.R.Chaurasia, Indian J. Pharm., 31, 81 (1969).
105. M.Ichikawa and H.Ichibagase, Chem. Pharm. Bull., 17, 2384 (1969).
106. C.Rufer, Justus Liebigs Ann. Chem., 726, 145 (1969).
107. E.F.Elslager, Z.B.Gavrilis, A.A.Phillips and D.F.Worth, J. Med. Chem., 12, 357, 591, 363 (1969).
108. E.F.Elslager, D.B.Capps and D.F.Worth, ibid, p. 597.
109. K.Prabhakaran, E.B.Harris and W.F.Kirchheimer, J. Bacteriol., 100, 935 (1969).
110. C.Shepard, Proc. Soc. Exp. Biol. Med., 132, 120 (1969).
111. W.A.Vischer, Arzneim.-Forsch., 18, 1529 (1968).
112. E.F.Godefroi, J.Heeres, J.van Cutsem and P.A.J.Jannsen, J. Med. Chem., 12, 784 (1969).
113. T.Zolnai, Arzneim.-Forsch., 19, 558, 1319 (1969).
114. M.Schoor, W.Duerckheimer, P.Klatt, G.Laemmler, G.Nesemann and E. Schrinner, ibid, p. 1807.
115. H.H.Freedman, A.E.Fox and R.S.Willis, Proc. Soc. Exptl. Biol. Med., 129, 796 (1969).
116. A.E.Fox and H.H.Freedman, Bacteriol. Proc., 1969, 99.
117. I.R.Gucklhorn, Mfg. Chem., 40, (6) 23 (1969).
118. R.Metzner, Pharm. Praxis, 1969, 218.
119. R.Patch and C.Hoehne, Zbl. Bakt. Parasitenk. Infektionskr. Hyg., Abt. 1 Orig., 211, 81, 88 (1969); Ringdoc#01690K and #01691K, Derwent Publ. Inc., 1970.
120. H.C.Ansel, W.P.Norred and I.L.Roth, J. Pharm. Sci., 58, 836 (1969).
121. N.M.Molnar, J. Soc. Cosmet. Chem., 20, 103 (1969).
122. M.Rotmistrov, I.O.Vassilevskaya, S.S.Stavskaya and N.F.Moghilevich, Mikrobiologiya, 38, 624 (1969).
123. T.Rutherford and J.G.Black, Brit. J. Dermatol., 81, Suppl. 4, 75 (1969).
124. S.Tenenbaum and D.L.Opdyke, Food Cosmet. Toxicol., 7, 223 (1969).
125. M.H.Zakaria and D.Taber, J. Med. Chem., 12, 707 (1969).
126. Y.Hamada, F.Tomita and H.Matuoka, Yakugaku Zasshi, 89, 1218 (1969).
127. N.A.Mukhina, R.Y.Galanova, G.Y.Kivman and E.A.Rudzit, Farmakol. Toksikol. (Moscow), 32, 449 (1969).
128. T. Ujiie, Chem. Pharm. Bull., 16, 2488 (1968).
129. M. Carissimi, P.G.DeMeglio, F.Ravenna and G.Riva, Farmaco, Ed. Sci., 24, 478 (1969).
130. A.J.Fritsch, C.E.Moore, T.S.Meyer, I.L.Shklair and L.F.Devine, Appl. Microbiol., 18, 684 (1969).
131. V.G.Stetsynk, Mikrobiol. Zh. (Kiev), 31, 417 (1969).
132. S.Watanabe, Bull. Chem. Soc., 42, 1152 (1969).

133. W.B.Hugo and M.Frier, Appl. Microbiol., 17, 118 (1969).
134. A.Doppstadt, H.C.Stark, H.G.Stoll and H.K.Grubmeueller, Arzneim.-Forsch., 19, 1764 (1969).
135. J.P.Paolini, L.J.Lendvay and F.P.Palopoli, J. Med. Chem., 12, 701 (1969).
136. F.Kobayashi and M.Tokunaga, Jap. J. Microbiol., 13, 29 (1969).
137. G.Franchi and P.Vigni, Farmaco, Ed. Sci., 24, 893 (1969).
138. T.N.Srivastava and S.K.Tandon, J. Prakt . Chem., 311, 878 (1969).
139. H.M.Butler, A.Hurse, E.Thursky and A.Shulman, Aust. J. Exp. Biol. Med. Sci., 47, 541 (1969).
140. A.Shulman, I.K.Reid, G.M.Laycock, S.Dixson and F.P.Dwyer, Aust. J. Exp. Biol. Med. Sci., 47, 203 (1969).
141. R.J.Bromfield, R.H.Dainty, R.D.Gillard and B.T.Heaton, Nature, 223, 735 (1969).
142. G.A.Troyan, P.K.Krichinyuk and G.K.Palii, Antibiotiki (Moscow), 14, 923 (1969).
143. H.Kourai and K.Takeichi, Hakko Kogaku Zasshi, 47, 603 (1969).
144. K.Yagishita and Y.Nagai, Yakugaku Zasshi, 89, 1152 (1969).
145. Y.Abe and S.Osanai, J. Amer. Oil Chem. Soc., 46, 365 (1969).
146. L.A.Balykova, A.F.Sobol, V.E.Limanov, L.M.Vorontsova and E.K. Skvortsova, Khim. Farm. Zh., 3, (1) 23 (1969).
147. J.Couquelet, J.Couquelet, J.B.Boyer, R.Cluzel and P.Toache, Chim. Ther., 4, 127 (1969).
148. A.F.Novak, J.M.Solar, R.R.Mod, F.C.Magne and E.L.Skau, Appl. Microbiol., 18, 1050 (1969).
149. S.B.Lodh and P.R.Pal, Sci. Cult., 34, 455 (1969).
150. J.T.Hill and F.W.Dunn, J. Med. Chem., 12, 737 (1969).
151. W.A.Zygmunt and T.A.Martin, J. Med. Chem., 12, 953 (1969).
152. B.Prescott, G.Lones and G.Caldes, Antimicrobial Agents and Chemotherapy-1968-p. 262.
153. B.A.Arbuzov, Khim. Farm. Zh., 3, (10) 9 (1969).
154. T.Takahashi, Y.Hamada, I.Takeuchi and H.Uchiyama, Yakugaku Zasshi, 89, 1260 (1969).
155. G.Casini, F.Claudi, M.Felici, M.Ferappi and M.Grifantini, Farmaco, Ed. Sci., 24, 732 (1969).
156. S.Okada and O.Tamemasa, Chem. Pharm. Bull., 17, 1432 (1969).
157. H.Schoenenberger, T.Bastug and D.Adam, Arzneim.-Forsch., 19, 1082 (1969).
158. G.M.Cingolani, F.Gualtieri and M.Pigini, J. Med. Chem., 12, 531(1969).
159. R.C.Tweit, R.D.Muir and S.Mizuba, ibid, p. 343.
160. W.G.Duncan and D.W.Henry, ibid, p. 25.
161. E.F.Elslager and D.F.Worth, ibid, p. 955.
162. A.Simek, A.Capek, J.Turinova, J.Weichet and J.Leiner, Folia Microbiol. (Prague), 14, 508 (1969).
163. H.Goeth and A.Wildfeuer, Arzneim.-Forsch. 19, 1000 (1969).
164. V.K.Chadha and H.K.Pujari, Can. J. Chem., 47, 2843 (1969).
165. T.Takahashi, M.Takaya and Y.Maki, Yakugaku Zasshi, 89, 1516,1617(1969).
166. I.Yamamoto, Biochem. Pharmacol, 18, 1463 (1969).
167. P.Papini and G.Auzzi, Gazz. Chim. Ital, 99, 97 (1969).

Chapter 11. Antiviral Agents

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Introduction - During the period covered by this review, the New York Acad. of Sci. 2nd Conf. on Antiviral Substances was held. The annals of the conference will be published in 1970. The total information presented at this meeting was so pertinent to those interested in antiviral agents that it would be redundant to try to include any of the reports. A report on the Virus Inhibition, Chemotherapy, and Interferon Session of the 1st Intern. Congr. of Virology was published during this period.[1] A symposium was held on antiviral substances at the 6th Intern. Congr. of Chemotherapy in Japan. A survey of antiviral agents for 1968 was presented.[2] The reader is also referred to a series entitled, "Recent Antiviral Chemotherapy Publications" now included in most issues of Chemotherapy.

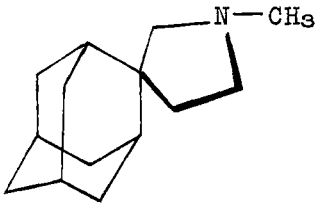
Stable Amines - Work continues to confirm the prophylactic and therapeutic activity of adamantanamine (amantadine HCl: Symmetrel) and α-methyl-1-adamantanemethylamine (rimantadine HCl) in reducing the severity of influenza A2 infections in humans. A controlled double-blind study utilizing contacts in a family environment indicated that adamantanamine reduced the spread of infection.[3] The incidence of clinical influenza as well as serological evidence of infection were reduced by treatment. Insomnia in one family was the only adverse symptom noted. In another study, patients reporting within 24 hours of the first signs of influenza were used to study the therapeutic activity of adamantanamine and α-methyl-1-adamantanemethylamine.[4] These investigators observed an increase in the rate of overall clinical improvement with shorter periods of fever, and an increase in the rate of disappearance of signs and symptoms of illness. No influence on antibody levels or side-effects were observed due to drug treatment. In another therapeutic trial, patients were treated within the first 48 hours of symptoms with α-methyl-1-adamantanemethylamine or placebo under double-blind conditions.[5] A shorter course of influenza A2 disease was observed with the nine treated patients. Since this was a prison study, it was impossible to evaluate the ability of drug-treated individuals to return to work sooner than placebo-treated individuals. Adamantanamine was given to incapacitated old people without significant side-effects, and although reduction of clinical influenza was not statistically significant, the results suggest that the drug had a prophylactic value.[6]

An extensive trial involving 2,554 men in the treated groups and 2,575 men in the control groups was made, but no influenza disease occurred in this population during this study.[7] No adverse side-effects were observed during the 20-day period of drug treatment.

The animal toxicological and pharmacological properties of adamantanamine have been described in detail.[8] Both cyclooctylamine and adamantanamine inhibited the growth of a 1969 clinical isolate of influenza A2 in tissue culture when the drug was present before infection.[9] Rous and Esh sarcoma virus multiplication in tissue culture is inhibited by α-methyl-1-adamantanemethylamine.[10] Adamantanamine and Rec.15/0209 in combination had synergistic anti-influenza activity in eggs but not in mice.[11]

A reexamination of the mode of action of adamantanamine indicates that with fowl plaque virus in chick embryo cells, the compound does not alter penetration but blocks uncoupling of virus particles.[12]

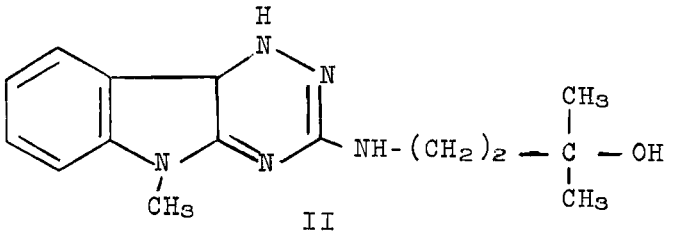
The search continues for adamantanamine analogs with more desirable properties. N-methyl-adamantane-2-spiro-3'-pyrrolidine (I) is probably of most interest.[13] This new derivative was observed to have a higher degree of activity and a higher therapeutic ratio than adamantanamine in experimental influenza infections in mice. In addition, a broader spectrum was observed including the inhibition of a strain of rhinovirus in tissue culture. In an attempt to increase basicity 1-adamantyl guanidine was synthesized.[14] The compound is protective in mouse influenza infections, but not more so than adamantanamine. The compound, 4-azahomoadamantane, afforded similar biological results.[15] A series of adamantane heterocyclic compounds has been described.[16] A protective effect against Asian A2 influenza in mice was reported for rac-endo-2-bornanamine.[17] A study of 41 analogs did not produce improved activity.



I

Thiosemicarbazones - No clinical experiments utilizing isatin thiosemicarbazones in poxvirus infections were reported during this period. The finding that isatin thiosemicarbazones active against poxvirus also inhibit the multiplication of certain strains of rhinovirus led to the finding that 2-methyl-4 [(5-methyl-5H-as-triazino- [5,6-b] indol-3-yl)amino] -2-butanol (II) was active on all rhinovirus strains tested in

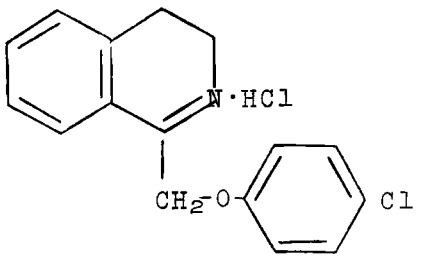
tissue culture; the ratio of a well-tolerated dose to minimum inhibitory concentration was at least five.[18] The compound is orally absorbed by the mouse, and it protects chimpanzees and gibbons infected with certain strains of rhinoviruses.[19]



II

A series of N-acyl and N-acyloxymethyl isatin thiosemicarbazones was synthesized in an attempt to expand the spectrum.[20] These compounds were not inhibitors of influenza WSN or rhinovirus strains. N-methylisatin-β-4':4'-dibutylthiosemicarbazone was shown to be an inhibitor of poliovirus RNA synthesis.[21] The compound directly inhibits cell-free polio RNA polymerase.

Isoquinolines - Two volunteer challenge studies using influenza virus B/ENG/13/65 indicated that 1-(p-chlorophenoxymethyl)-3,4-dihydroisoquinoline HCl (III) given either orally or intranasally suppressed symptoms and signs of infection.[22] The daily oral dose was 1 g/day while the intranasal dose was only 2.16 mg/day. No evidence of toxicity was seen. During a A2/Hong Kong/68 epidemic 1-(p-chlorophenoxymethyl)-3,4-dihydroisoquinoline was evaluated as a prophylactic agent.[23] The drug administered as nose drops appeared to the authors to have no therapeutic effect during the first three days, but it may have prophylactically reduced the incidence of influenza after three days.

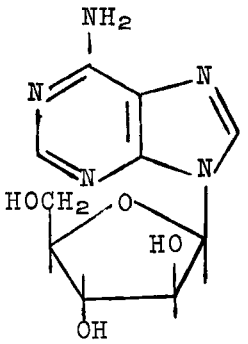


III

Tissue culture studies with 1-(p-chlorophenoxymethyl)-3,4-dihydroisoquinolines indicated that the compound inhibits influenza A virus only when virus and compound are mixed befor and during virus adsorption.[24] Effectiveness was dose-dependent; longer duration and higher temperature of incubation increased activity, but the compounds appeared to be acting by preventing some postpenetration step. The structure activity relationships of a series of 1-phenoxymethyl-3,

4-dihydroisoquinolines indicated severe steric limitations when measured by ability to inhibit viral neuraminidase.[25] The inhibition was noncompetitive. Modification of 1-phenoxymethyl-3,4-dihydroisoquinoline did not lead to a great increase in activity. A Hansch analysis led to the conclusion that optimum activity had been achieved and also indicated that there was a correlation between enzyme inhibition, the hydrophobic constant, and the dipole moment.[26]

Nucleosides - The therapeutic activity of 9-β-D-arabinofuranosyladenine (ara-A, IV) against herpes simplex virus mouse infections was demonstrated.[27] Protected treated mice could not be reinfected and virus could not be recovered from treated mice 21 days postinfection. Virus from treated mice did not appear to have developed resistance. Ara-A inhibits the growth of cytomegalovirus in tissue culture only when ara-A is present during the growth cycle.[28] This is in contrast to thioguanine which inhibits growth by pretreatment of cells.[28] The spectrum of ara-A was extended to include myxoma and pseudorabies virus.[29] Ara-A activity against pseudorabies virus was not altered by addition of excessive adenosine, deoxyadenosine, guanosine, deoxyguanosine, or thymididine in tissue culture. Ara-A given systemically therapeutically prevented keratitis and death from encephalitis caused by herpesvirus in rabbits.[30] There was no indication of cross resistance with 5-Iododeoxyuridine (IUDR).



IV

Two reports described the successful systemic treatment of herpes encephalitis patients with IUDR.[31,32] Only transient and mild toxic effects were observed. It was suggested that although true genetic resistance to IUDR develops easily with herpesvirus, resistance did not appear to be a major factor in recurrent herpetic keratitis.[33] Recurrent herpetic keratitis is probably due to a chronic infection of the lacrimal gland and conjunctiva.

Nucleosides containing a carbon ribose linkage from fermentations such as formycin, showdomycin, and pyrazomycin, are of interest as a potential type of metabolically stabilized antivirals. Formycin inhibits the growth of a wide spectrum of DNA and RNA viruses.[34] In Vero cells infected with Japanese encephalitis virus, RNA synthesis was markedly

inhibited, protein synthesis slightly inhibited, while DNA synthesis was not altered. Although the mechanism of the antiviral action of formycin was not elucidated, the effective replacement of adenine nucleotides by formycin species in a variety of biochemical reactions was established.[35] Methods of synthesis of pyrazolo[4,3-d]pyrimidine derivatives as a synthetic approach to formycin B have been described.[36] The *in vitro* and *in vivo* antivaccinia activity of pyrazomycin was reported.[37]

A comparison of 1-β-D-arabinofuranosylcytosine and 1-β-D-arabinofuranosyl-5-fluorocytosine as inhibitors of herpes keratitis infection in rabbits and of vaccinia in tissue culture indicated comparable activity.[38] The compound, 5-ethyldeoxyuridine, was synthesized and considered nonmutagenic but showed antivaccinia activity in tissue culture equivalent to IUDR.[39] It was suggested that substitution in the 5'-position may be a general approach to separate these biological activities. 5-Azacytidine showed an inhibitory effect on Eastern equine encephalitis virus in chick embryo cells.[40] The lability of viral RNA synthesis in the presence of 5-azacytidine was increased. A study of a series of branched chain sugar nucleosides indicated that branching in the 3'-position, i.e., 3'-C-methylcytidine, produced compounds which were effective in suppressing vaccinia virus multiplication as measured by the tail lesion assay.[41] The synthesis of novel nucleosides as potential antiviral agents was described.[42] Halogenated derivatives of tubercidin were synthesized[43] and 5-bromotubercidin was shown to stimulate viral growth while simultaneously inhibiting host cell macromolecule synthesis.[44]

Other Synthetic Antivirals - Clinical investigations in man have indicated that 1-allyl-3,5-diethyl-6-chlorouracil applied topically as a 1% solution is effective in the treatment of all types of recurring herpetic skin and mucous diseases including herpes genitalis and stomatitis aphthosa.[45] The compound was first found to be active in tissue culture and then in rabbits. A study of the virostatic properties of 1-allyl-3,5-diethyl-6-chlorouracil indicated that this type of compound exerts its action through its allylic double bond and does not interfere with nucleic acid metabolism.[46]

Two 4-diethylaminoalkylamino derivatives of 7-chloro-2-styrylquinoline protected mice against Semliki Forest virus infections.[47] During the synthesis and study of antiviral activity of sulfonamidopropionamidine derivatives, 3-(4-ethyl-benzene-sulfonamido)propionamidine HCl was found to have activity against experimental mouse influenza infections.[48] Four series of heterocyclic substituted ureas have been described that are active *in vivo* against virus infections

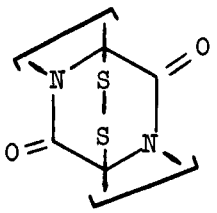
by injections but not orally.[49] The structure-activity relationships were evaluated in terms of the ED_{50} values obtained in Coxsackie A21 mouse infections. The 1-propyl derivative of hydroxybenzyl benzimididazole showed an indication of in vivo activity against Coxsackie A9 virus in mice.[50] Corticosteroids increased the yield of hepatitis virus as well as the severity of symptoms in mice.[51] The animal toxicology and human tolerance for the antiherpetic drug, 4'-[2-nitro-1-(p-tolythio)ethyl]acetanilide have been reported.[52]

Virucidal activity was found to be a property of 2,6-dialkoxypyranes.[53] Highest specific activity was shown by 2,6-dibutoxy-3-methyl-Δ^3-dihydropyran, but no systemic activity was measured using mouse influenza infections. Canavanine inhibited growth of Semliki Forest virus in tissue culture.[54] The O-carboxyquinone and O-hydroxyquinone derivatives of triphenylmethane were found to prevent viral RNA attachment to ribosomes in a cell-free system.[55] Further studies on the structure-activity relationships of hydroxybenzyl benzimidazole have defined the structure requirements for selective inhibition.[56] Nonsteroidal anti-inflammatory agents were compared for in vitro antiviral activity and for activity in several biochemical tests used for finding anti-inflammatory drugs.[57] With known compound, there was a positive correlation. The compound, α-methyl-2-phenoxathiin methanol was found to be an effective inhibitor of poliovirus; however, rapid development of drug-dependent variants to the drug was observed.[58] The compound, N_1-isonicotinoyl-N_2-3-methyl-4-chlorobenzoylhydrazine inhibited the release of vaccinia virus in tissue culture but had no inhibitory action on vaccinia growth in mice or rabbits.[59]

Antiviral Antibiotics - Screening fermentation mixtures, identifying known compounds, and assaying samples during the purification of active fermentation products, are considered to be more difficult than the examination of pure synthetic compounds as antiviral agents. However, a review of the development of useful antibacterials would indicate that the increased effort is justified. While most antibiotics isolated as antibacterial substances will eventually be tested in antiviral systems, it seems valid to expect certain cultures to produce specific antivirals which are not inhibitors of other microorganisms. In a series of papers on antiviral and antitumor antibiotics, Takatsuki et al., have presented some correlations between antiviral antibiotics and numerous other parameters.[60] Such correlations are quite frequently made, but seldom reach publication.

Rifampicin, an antibacterial antibiotic known to be a specific inhibitor of bacterial but not of mammalian DNA-dependent RNA synthesis, was shown to be an inhibitor of vaccinia virus multiplication in tissue culture.[61,62] It should be noted that the concentration of rifampicin required to inhibit vaccinia virus multiplication (60 μg/ml) is high in relation to the amount needed to inhibit S. aureus (0.005 μg/ml) or E. coli (20 μg/ml). Rifampicin also inhibits focus formation in chick fibroblasts infected with Rous sarcoma virus.[63] Rifampicin prevents the assembly of DNA and proteins into mature virus particles.[64] These authors also suggested that rifampicin does not inhibit the DNA-dependent RNA polymerase associated with the vaccinia particle which is already combined with template. More recent work indicates that rifampicin when added early to the growth cycle inhibits the formation of functional particulate RNA polymerase activity.[65] Whether this enzyme is essential to the assembly of vaccinia virus is not known.

Antiviral antibiotics containing the epidithiapiperazinedione ring system (V) are receiving further attention. Oryzachlorin was reported as a new member of the group and shown to have in vitro antiviral and antifungal activity.[66] Studies to examine the mode of action of aranotin and related metabolites indicated that viral RNA synthesis is inhibited.[67] Studies utilizing purified enzymes revealed that the selective activity was attributed to the inhibition of RNA-dependent RNA polymerase activity without inhibiting DNA-dependent RNA polymerase activity. Gliotoxin was also shown to inhibit viral RNA synthesis.[68] In contrast, however, in studies with isolated RNA-dependent RNA polymerase, gliotoxin was considered a less effective inhibitor as judged by an arbitrary comparison of respective concentrations; a more realistic comparison of gliotoxin and aranotin might be based on the ratio of molecules required per molecule of enzyme.



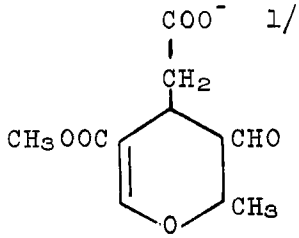
V

The in vitro antiviral activity and lack of in vivo activity against non-oncogenic viruses of mycophenolic acid was confirmed.[69] The antiviral tissue culture activity of mycophenolic acid was attributed to antagonism of the GTP requirement for virus multiplication.[70] Mycophenolic acid was shown to inhibit the conversion of hypoxanthine to guanine nucleotides.[71]

Distamycin A inhibits vaccinia and herpesvirus in tissue culture and exerts a marked inhibitory activity against Shope's fibroma in rabbits.[72] Distamycin inhibits the template activity of DNA for DNA-polymerase by interacting with the template DNA.[73] Daunomycin also interacts with DNA and inhibits DNA but not RNA viruses.[74] The topical use of daunomycin for IUDR-resistant virus was suggested.

Streptothricins were shown to inhibit influenza in eggs and mice and the degree of activity appeared to be linearly related to the β-lysine content.[75] The activity of julimycin was attributed to an action of stimulating a host-defense mechanism not involving interferon.[76] Paecilomycerol, a steroidal antibiotic, was shown to have antiviral activity without antibacterial or antifungal activity.[77]

Natural Products - Calcium elenolate (VI) was isolated from acid hydrolyzed aqueous extracts of various parts of olive plants and shown to be virucidal for a wide variety of viruses.[78] There was loss of influenza infectivity without a change in HA titer or neuraminidase activity. The compound did not inactivate infectious RNA. The virucidal activity was reversed or removed by adding certain amino acids. Calcium elenolate was shown to inhibit parainfluenza 3 virus multiplication in an intranasal infection in hamsters when the drug was given intranasally.[79] Although it appears that calcium elenolate binds nonspecifically to amino acids, animal safety studies indicated that there is some specificity for the virus and that an antiviral activity may be obtained without toxicity to the nasal passage.[80]



VI

Antivirin, a new antiviral agent produced by cultured cells, is not degradable by proteolytic enzymes and does not possess species specificity.[81] Antivirin inhibits viral RNA synthesis without altering cellular RNA synthesis.[82] An antiviral principle is produced by tomato plants infected with TMV. The course of the virus disease was altered in the producing plant and in other plants.[83] A water extract of the leaves of the Margosa tree produced a crude mixture that is virucidal for vaccinia virus and variola, the only viruses tested.[84] A screening program of Japanese-Chinese medicinal plants for antiviral agents has led to the discovery of two substances showing protective effects in experimental mouse

infections.[85] L-asparaginase was shown to inhibit splenomegaly and prolong survival time in Rauscher leukemia virus-infected mice.[86]

Interferon - Interferon is the mediator of the host-response mechanism studied extensively in attempts to inhibit a broad spectrum of viruses. Emphasis is currently being directed toward finding inducers of circulating interferon rather than the utilization of purified exogenous interferon alone. For a general review on interferon, Vilcek's recent monograph[87] is suggested. The "Interferon Scientific Memoranda" provides a rapid communication service for scientists working in interferon research.[88]

The elucidation of the mode of action and composition of two natural inducers, statolon and helenine, led to studies of polynucleotides as interferon inducers. Double strandedness is considered an absolute requirement for interferon induction by nucleotide homopolymers by some workers,[89] but not by others.[90] The specific activity of a wide variety of polynucleotides with double-stranded structure is higher than either single-stranded or triple-stranded structures.[91] Pairs of different polynucleotides differ widely in specific activity indicating that a stable secondary structure may be required.[92] Present data suggests that ribopolynucleotide pairs are much more active than pairs of mixed composition which in turn are more active than deoxyribopolynucleotide pairs.[91] Substitution of thiophosphate for phosphate in poly rA:poly rU resulted in an increase in the efficacy of *in vitro* and *in vivo* interferon induction and a proportional increase in the resistance to ribonuclease action.[93] The finding that double-stranded RNA isolated from a virus in a strain of *P. chrysogenum*, a commercial penicillin-producing strain, was an efficient inducer of interferon provides a potential large supply of natural double-stranded RNA.[94]

Polymers other than polynucleotides also have been found to induce interferon. Pyran-2-succinic anhydride, 4,5-dicarboxytetrahydro-6-methyl-anhydride polymers in low daily doses protect mice infected with lethal doses of Friend or Rauscher leukemia virus.[95] A correlation between degree of protection and interferon levels was noted. Vinyl sulfate polymers[96] and divinyl ether-maleic acid copolymers[97] induce interferon and protect mice against lethal virus infections. Both of these substances had activity that could not be accounted for by interferon alone. The dependency on molecular weight of polyacrylic acid polymers for interferon induction indicated a parallel dependency for toxicity.[98] "Chlorite oxidized oxyamylose" was shown to protect mice from lethal Mengo, Semliki Forest, and influenza PR8A virus infections and to reduce

lesions from vaccinia virus infection, probably by the induction of interferon.[99] An RNA:polysaccharide complex has been isolated from Cunninghamella blakesleeana and was found to enhance host resistance to virus infection.[100] Only the complex was active in vitro, but the complex and both its components were active in vivo. Interferon was detected only in in vitro studies. A mushroom extract has been described that induces interferon and also protects mice from influenza infections when given parenterally.[101] Desmyter[102] has reported that kanamycin, 6-amino-6-deoxy-D-glucose, and 3-amino-3-deoxy-D-glucose produce an interferon-like inhibitor upon administration to chickens.

As is true in the search for all antiviral agents, finding active agents is not the problem, but finding an inducer that is efficient at a level which can be used without danger of some sort of toxicity is the problem. Hilleman[103] states that all known inducers of interferon except double-stranded RNA are so toxic that they cannot be considered for human therapy. Although double-stranded RNA was considered nontoxic, numerous reports have indicated toxicity. For example, poly rI:poly rC is pyrogenic in rabbits,[104] possesses toxicity similar to that exhibited by endotoxin,[105] and is associated with embryotoxic effects in the rabbit.[106] The test for embryotoxic effect might be useful in initial studies of toxicity of new inducers. Leonard et al.[107] after evaluating the toxicity of poly rI:poly rC, sugar beet virus RNA, and Pseudomonas phage RNA suggested that double-stranded RNA is intrinsically toxic and that thymic atrophy, lymphocyte transformation, and interferon production are linked.

The route of administration of an interferon inducer may be important both for activity and toxicity.[108] Most interferon inducers are inactive against influenza virus infections, unless the inducer is given intranasally. This route may be effective against many viruses and may reduce toxicity.

Methods - Since a large variety of clinically active and inactive compounds have not been established, the optimum methods of finding or evaluating new active compounds remain unknown. The development of new methods based on a wide variety of objectives is very important.

Interest and need is especially high with rhinoviruses. There are so many strains of rhinoviruses that chemotherapy would appear to be the method of choice for prevention or treatment. An efficient system for plaquing most rhinovirus strains has been developed utilizing a special line of HeLa cells.[109] Because of the need of a broad-spectrum agent for rhinoviruses, it has been suggested to screen in tissue culture tests with mixtures of strains of rhinoviruses.[110]

Methods of infecting gibbons with rhinovirus Type 2 were described. Infected gibbons (7 of 8) shed virus for up

to 8 days and all animals showed seroconversion.[111] Vervet monkeys were infected with equine rhinovirus but were not susceptible to infection with human rhinovirus IB.[112] Equine rhinovirus is probably a rhinovirus but differs in properties from human rhinovirus. Organ cultures were shown to be better for the isolation of rhinoviruses while standard cell cultures were more effective for other viruses.[113] Immunodiffusion technics were adapted for detecting and titering rhinovirus antibody in isolations from experimental animals.[114]

A sensitive screening system was developed using non-fatal influenza infections followed by animal titrations of lung homogenates of treated and control animals.[115] A screening test utilizing the phonocardiograph, which measures degree of infection by evaluating frequency of rales, was described.[116] During the examination of a single series of compounds for anti-influenza activity, 20 of 230 compounds passed a lung congestion assay, and 3 of 20 passed a second test based on number of survivors.[117] A method was studied to determine the effect of an antiviral substance on the experimental spread of influenza.[118] The utility of measurement of resistance to air flow across the turbinates and increase in number of nasal leukocytes in influenza-infected ferrets was validated.[119] Respiratory syncytial (RS) and Reo Type I virus were also used. The course, extension, and transmission of RS infection of baby hamsters can be followed by plaque assays of daily nasal washes.[120] Rapid diagnosis of RS and parainfluenza by electron microscopy was suggested.[121]

The methods used for the vaccinia virus tail lesion assay were applied to vesicular stomatitis and herpes simplex viruses.[122] Methods of utilizing drug and disease pooling for screening tests were described.[123] Attempts were made to correlate *in vitro* and *in vivo* methods for the study of virus chemotherapeutic agents using known compounds with a known history.[124] The use of marmosets in virological research was described including studies with human infectious hepatitis virus.[125]

A method of looking for specific inhibitors for RNA-dependent RNA polymerase was employed in the detection of a new compound.[126] A rapid plaque assay utilizing hemadsorption for rubella virus has been developed.[127] Tissue culture assays utilizing a continuous concentration gradient gave a direct indication of tissue culture therapeutic ratio.[128] Dye uptake methods for assessing viral cytopathogenicity have been quantitated.[129] An *in vitro* technique was developed for the detection of Marek's disease virus.[130]

Methods of studying the *in vitro* and *in vivo* antiviral activity of virucidal agents were described.[131]

References

1. "International Virology I", Proceedings of the 1st International Congress for Virology (Helsinki) 1968. Ed. J.L.Melnick (Karger Basel/New York, 1969). V.M.Zldanov and M.R.Hilleman, p. 88 (1969).
2. C.E.Hoffmann, Ann.Repts.Med.Chem.,1968, p. 117 (1969).
3. A.W.Galbraith, J.S.Oxford, G.C.Schild and G.I.Watson, Lancet, 2, 1026 (1969).
4. W.L.Wingfield, D.Pollack and R.R.Grunert, New Eng.J.Med., 281, 579 (1969).
5. S.Rabinovich, J.T.Baldini and R.Bannister, Amer.J.Med.Sci., 257, 328 (1969).
6. J.T.Leeming, Brit.Med.J., 1, 313 (1969).
7. Sixth International Congress of Chemotherapy (Japan), Aug. 10-15 (Abstracts) R.O.Peckinpaugh, F.B.Askin, W.E.Pierce and G.G.Jackson, p. 303 (1969).
8. V.G.Vernier, J.B.Harmon, J.M.Stump, T.E.Lynes, J.P.Marvel and D.H.Smith, Toxicology and Applied Pharm., 15, 642 (1969).
9. J.F.Greene,Jr., B.E.Tilton and J.J.Quilligan,Jr., Appl.Microb., 18, 533 (1969).
10. A.M.Wallbank, Proc.Soc.Exptl.Biol.Med., 131, 1025 (1969).
11. P.Altucci, G.L.Varone and F.Magrassi, Chemotherapy, 14, 140 (1969).
12. N.Kato and H.J.Eggers, Virology, 37, 632 (1969).
13. A.Peters, C.A.deBock, G.B.Paerels and J.L.M.A.Schlatmann (Ref.7) p.306.
14. H.W.Geluk, J.Schut and J.L.M.A.Schlatmann, J.Med.Chem., 12, 712 (1969).
15. J.G.Korsloot, V.G.Keizer and J.L.M.A.Schlatmann, Rec.Trav.Chim.Pays-Bas, 88, 447 (1969).
16. T.Sasaki, S.Eguchi and T.Toru, Bull.Chem.Soc.Jap., 42, 1617 (1969).
17. W.Mosimann, J.Borgulya and K.Bernauer, Experientia, 25, 726 (1969).
18. J.M.Z.Gladych, J.H.Hunt, D.Jack, R.F.Haff, J.J.Boyle, R.C.Stewart and R.J.Ferlauto, Nature, 221, 286 (1969).
19. R.Haff (Ref. 7) p. 463.
20. C.Runti and F.Collino, Farmaco (Pavia) Ed.Sci., 24, 577 (1969).
21. G.D.Pearson and E.F.Zimmerman, Virology, 38, 641 (1969).
22. P.N.Meenan and I.B.Hillary, Lancet, 2, 614 (1969).
23. P.N.Meenan and I.B.Hillary, ibid, 641 (1969).
24. D.Hobson, H.I.Flockton,and M.G.Gregory, Brit.J.Exp.Path., 50, 494 (1969).
25. M.S.Tute, K.W.Brammer, B.Kaye and R.W.Broadbent, J.Med.Chem., 13, 44 (1970).
26. M.S.Tute, ibid, 48 (1970).
27. Ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington D.C. (Oct. 27-29), Abstracts, F.A.Miller, B.J.Sloan and C.A.Silverman, p. 10 (1969).
28. R.W.Sidwell, G.Arnett, G.J.Dixon, and F.M.Schabel,Jr., Proc.Soc.Exptl. Biol.Med., 131, 1223 (1969).
29. R.W.Sidwell, G.Arnett and F.M.Schabel,Jr., (Ref. 7) p. 298
30. H.E.Kaufman, E.D.Ellison and W.Townsend, Interferon Scientific Memoranda, Memo 305/1 (1969).
31. J.H.Adams, W.B.Jennett and J.D.Miller, Lancet, 1, 987 (1969).
32. M.Rappel, ibid, 2, 272 (1969).
33. H.E.Kaufman (Ref. 7) p. 464.

34. C.Nishimura and H.Tsukeda (Ref. 7) p. 293
35. D.C.Ward, A.Cerami and E.Reich, J.Biol.Chem., 244, 3243 (1969).
36. M.Sprinzl, J.Farkas and F.Sorm, Tetrahedron Lett., 5, 289 (1969).
37. F.Streightoff, J.D.Nelson, J.C.Cline, K.Gerzon, R.H.Williams and D.C.DeLong (Ref. 27) p. 8.
38. H.N.Prince, E.Grumberg, M.Buck,and R.Cleeland, Proc.Soc.Exptl.Biol. Med., 130, 1080 (1969).
39. M.Swierkowski and D.Shugar, J.Med.Chem., 12, 533 (1969).
40. S.Halle and A.Brown, Bact.Proc., p. 161 (1969).
41. E.Walton, S.R.Jenkins, R.F.Nutt and F.W.Holly, J.Med.Chem., 12, 306 (1969).
42. I.M.Downie, J.B.Lee, T.J.Nolan and R.M.Allen, Tetrahedron, 25, 2339 (1969).
43. B.C.Hinshaw, J.F.Gerster, R.K.Robins and L.B.Towsend, J.Heterocyclic Chem., 6, 215 (1969).
44. E.Reich, A. Symposium on "Recent Advances in Nucleoside Chemistry" at the 156th National Meeting of the American Chemical Society, Atlantic City, N.J., 1968, MEDI 30.
45. K.K.Gauri and B.Rohde, Klin.Wschr., 47, 375 (1969).
46. K.K.Gauri and H.Kohlhage, Chemotherapy, 14, 158 (1969).
47. J.C.Chan and H.H.Gadebusch, Experientia, 25, 329 (1969).
48. T.Ueda, K.Nagahara, K.Takahashi and S.Sato, Chem.Pharm.Bull., 17, 2065 (1969).
49. C.J.Paget, C.W.Ashbrook, R.L.Stone and D.C.DeLong, J.Med.Chem., 12, 1097 (1969).
50. D.G.O'Sullivan, D.Pantic, D.S.Dane, M.Briggs, Lancet, 1, 446 (1969).
51. D.V.Datta and K.J.Isselbacher, Gut, 10, 522 (1969).
52. E.Novak, C.A.Schlagel and G.Elliott, Toxicology and Appl. Pharm., 14, 89 (1969).
53. G.N.Pershin, N.S.Bogdanova and S.M.Machin, (Ref. 7) p. 300.
54. M.Ranki and L.Kaariainen, Scand.J.Clin.Lab.Invest., 23, 61 (1969).
55. A.P.Grollman, Bull.N.Y.Acad.Med., 45, 481 (1969).
56. I.Tamm, H.J.Eggers, R.Bablanian, A.F.Wagner and K.Folkers, Nature, 223, 785 (1969).
57. A.D.Inglot, J.Gen.Virol., 4, 203 (1969).
58. J.D.Nelson, C.J.Paget, J.C.Cline, M.L.Bohnstadt, B.R.Warren and D.C. DeLong (Ref. 27) p.8.
59. N.Kato, H.J.Eggers and H.Rolly, J.Exp.Med., 129, 795 (1969).
60. A.Takatsuki, I.Yamaguchi, G.Tamura, T.Misato and K.Arima, J.Antibiotics, 22, 442 (1969).
61. E.Heller, M.Argaman, H.Levy and N.Goldblum, Nature, 222, 273 (1969).
62. J.H.Subak-Sharpe, M.C.Timbury and J.F.Williams, ibid, 341.
63. H.Diggelmann and C.Weissman, ibid, 224, 1277 (1969).
64. B.Moss, E.N.Rosenblum and K.Katz, ibid, 224, 1280 (1969).
65. B.R.McAuslan, Biochem.Biophys.Res.Comm., 37, 289 (1969).
66. A.Kato, T.Saeki, S.Suzuki, K.Ando, G.Tamura and K.Arima, J.Antibiotics, 22, 322 (1969).
67. D.C.DeLong, J.N.Nelson, J.C.Cline, N.Neuss and P.P.K.Ho (Ref. 7) p. 301.
68. J.K.McDougall, Archiv für die gesamte Virusforschung, 27, 255 (1969).
69. D.N.Planterose, J.Gen.Virol., 4, 629 (1969).

70. J.C.Cline, J.D.Nelson, K.Gerzon, R.H.Williams and D.C.DeLong, Applied Microbiology, 18, 14 (1969).
71. T.J.Franklin and J.M.Cook, Biochem.J., 113, 515 (1969).
72. Y.deRatuld and G.H.Werner (Ref. 7) p. 292.
73. B.Puschendorf and H.Grunicke, FEBS Letters, 4, 355 (1969).
74. A.Cohen, E.H.Harley and K.R.Rees, Nature, 222, 36 (1969).
75. K.I.Germanova and T.Ya.Goncharskaya, Antibiotiki., 14, 48 (1969).
76. K.Katagiri, S.Nishiyama and K.Sato (Ref. 7) p. 291.
77. A.Kato, K.Ando, T.Kimura, G.Tamura and K.Arima, J.Antibiotics, 22, 419 (1969).
78. H.E.Renis (Ref. 27) p. 6.
79. M.G.Soret (Ref. 27) p. 6.
80. G.A.Elliott and E.N.DeYoung (Ref. 27) p. 7.
81. Y.Seto and S.Toyoshima (Ref. 7) p. 15.
82. H.Tonegi, Y.Seto and S.Toyoshima (Ref. 7) p. 16.
83. K.C.Chadha and B.H.MacNeill, Can.J.Botany, 47, 513 (1969).
84. A.R.Rao, S.Sukumar, T.V.Paramasival, S.Kamalakshi, A.R.Parashuraman and Shantha, Ind.J.Med.Res., 57, 495 (1969).
85. E.Furusawa, S.Furusawa, M.Kroposki and W.Cutting (Ref. 7) p. 462.
86. W.F.Campbell and A.S.Levine, Life Sciences, 8, 1033 (1969).
87. J.Vilcek, "Interferon", Springer-Verlag Inc., New York, Monographs No. 6 (1969).
88. Interferon Scientific Memoranda, ARIES Corp., McLean, Virginia (Natl. Inst. Allergy and Infectious Diseases) 1969.
89. G.P.Lampson, A.A.Tytell, A.K.Field, M.M.Nemes and M.R.Hilleman, Proc. Soc.Exptl.Biol.Med., 132, 212 (1969).
90. A.Billiau, C.E.Buckler, F.Dianzani, C.Uhlendorf and S.Baron, ibid., 790 (1969).
91. C.Colby and M.J.Chamberlin, Proc.Natl.Acad.Sci., 63, 160 (1969).
92. E.DeClercq and T.C.Merigan, Nature, 222, 1148 (1969).
93. E.DeClercq, F.Eckstein and T.C.Merigan, Science, 165, 1137 (1969).
94. G.T.Banks, K.W.Buck, E.B.Chain, J.E.Darbyshire and F.Himmelweit, Nature, 223, 155 (1969).
95. M.A.Chirigos, W.Turner, J.Pearson and W.Griffin, Int.J.Cancer, 4, 267 (1969).
96. P.E.Came, M.Lieberman, A.Pascale and G.Shimonaski, Proc.Soc.Exptl. Biol.Med., 131, 443 (1969).
97. N.A.Miner and J.Koehler, Bact.Proc., p. 150 (1969).
98. J.F.Niblack, M.McCreary and N.L.Stone (Ref. 88) Memo 148/1.
99. A.Billiau, J.Desmyter and P.DeSomer (Ref. 88) Memo 300/1.
100. M.Y.Goore, J.F.Naylor, L.R.Fare, J.R.Valenta, W.B.Flagg, R.F.Haff, C.J.Ciruollo, J.P.Pagano and R.J.Ferlauto (Ref. 88) Memo 325/1.
101. A.Tsunoda, F.Suzuki, N.Sato and N.Ishida (Ref. 7) p. 466.
102. J.Desmyter (Ref. 1) p. 101.
103. M.R.Hilleman (Ref. 1) p. 100.
104. H.L.Lindsay, P.W.Trown, J.Brandt and M.Forbes, Nature, 223, 717 (1969).
105. M.Absher and W.R.Stinebring, ibid., 715 (1969).
106. R.H.Adamson and S.Fabro, ibid., 718 (1969).
107. B.J.Leonard, E.Eccleston and D.Jones, ibid., 224, 1023 (1969).
108. D.C.DeLong and W.J.Kleinshmidt (Ref. 7) p. 465.
109. R.M.Conant, D.C.Thomas and V.V.Hamparian, Bact.Proc., p. 197 (1969).

110. R.Haff (Ref. 7) p. 463.
111. C.A.Pinto and R.F.Haff, Bact.Proc., p. 180 (1969).
112. G.V.Martin and R.B.Heath, Brit.J.Exp.Path., 50, 516 (1969).
113. P.G.Higgins, E.M.Ellis and D.A.Woolley, J.Med.Microbiol., 2, 109 (1969).
114. S.Lipkin, J.J.Boyle and R.F.Haff, Bact.Proc., p. 198 (1969).
115. G.D.Mayer and R.F.Krueger (Ref. 7) p. 126.
116. M.Kaji, H.Tani and S.Kashiwagi (Ref. 7) p. 127.
117. S.Toyoshima, Y.Seto, H.Fujita, H.Tonegi, J.Abe, T.Watanabe and K. Fujimoto (Ref. 7) p. 302.
118. M.W.Rytel and J.L.Schulman, J.Gen.Virol., 5, 429 (1969).
119. R.F.Haff and C.A.Pinto (Ref. 7) p. 470.
120. R.W.Tankersley,Jr., Bact.Proc., p. 172 (1969).
121. J.H.Joncas, R.Williams, L.Berthiaume, P.Beaudry and V.Pavilanis, Lancet, 1, 956 (1969).
122. S.Yoshimura, R.T.Christian, G.D.Mayer and R.F.Krueger (Ref. 7) p. 128.
123. D.C.DeLong, L.A.Baker, and C.E.Redman (Ref. 7) p. 129.
124. H.E.Kaufman (Ref. 7) p. 469.
125. F.Deinhardt, Ann.N.Y.Acad.Sci., 162, 551 (1969).
126. I.Haruna, I.Watanabe, Y.Yamada and K.Nagaoka (Ref. 7) p. 460.
127. N.J.Schmidt. E.H.Lennette and J.Dennis, Proc.Soc.Exptl.Biol.Med., 132, 128 (1969).
128. A.German, Mme J.Panouse-Perrin et Mme Cl.Choisy, Proc.Probl.Pharm., 24, 453 (1969).
129. N.B.Finter, J.Gen.Virol., 5, 419 (1969).
130. A.Y.Elliott, D.E.Bordt and D.P.Gustafson, Bact.Proc., p. 164 (1969).
131. H.E.Renis (Ref. 7) p. 468.

Chapter 12. Antiparasitic Agents

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Introduction - The background of parasitism in today's world and the current status of antiparasitic chemotherapy has been outlined in the first four volumes of these reports. Information on new and potential compounds against human and animal parasitoses will be presented in the same chapter this year.

MALARIA

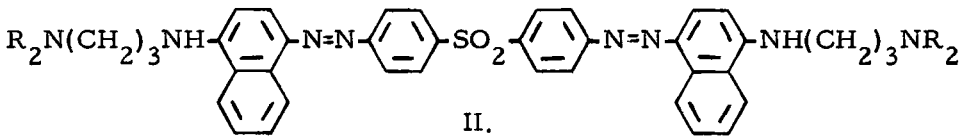
Preclinical screening and evaluation - Rodent malaria, chiefly Plasmodium berghei in mice continues to be the most popular system for primary screening. A few laboratories use the avian species P. gallinaceum in chickens and the use of P. chabaudi and P. vinckei in mice has been explored. For more detailed evaluation of compounds which appear promising in the primary screens simian malaria (usually P. cynomolgi or P. knowlesi) is employed. The progression of compounds through these types of animal models is a rough indication of the stage of their development toward the clinical phases of investigation.

Rodent malaria - The chemotherapy of Plasmodium berghei is the subject of an extensive review by Aviado[1] who is also engaged in a series of studies on pathologic physiology of P. berghei[2-6].

A number of compounds whose structures represent minor modifications of the 4-(substituted amino) group of chloroquine and hydroxychloroquine were made and tested[7,8,9]. None appeared to be more promising than the model compounds.

Six chloroquine derivatives[10] with unsaturation in the diamine side chain were found to be less toxic than chloroquine and some were more potent. The modifications were in the form of acetylenic and cis and trans ethylenic bonds.

I. PSBA



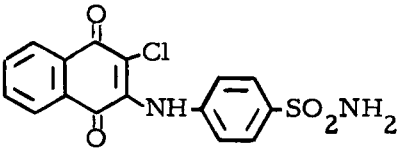
II.

The sulfones have continued to be of interest as prophylactic and repository drugs and numerous such compounds have been synthesized[11-13]. Notable are 4', 4'''-[p-phenylenebis(methylidyneimino-p-phenylenesulfonyl)]bisacetanilide, known as PSBA (I), and analogs[14].

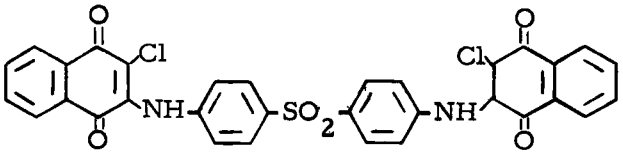
Attempts to combine the activity of the naphthylazo-sulfonamides with that of the sulfones resulted in N', N''-[sulfonylbis (p-phenyleneazo-1, 4-naphthylene)] bis(N', N'-dialkylalkylenediamines) (II) with marked activity but which were unable to overcome DDS resistance[15]. Of 24 diphenyl-sulfone derivatives 5 were active and 3 were curative[16]. Biguanide and aminourea derivatives of diphenyl sulfide, sulfoxide and sulfone were tested and three out of twelve were active[17].

1-Methyl-3-nitro-1-nitrosoguanidine (NMNG), which is antineoplastic in mice, and mitomycin C have an effect on the infectivity of P. berghei when parasitized blood is incubated with the compound and subsequently inoculated into previously uninfected mice[18]. The nitrosoguanidine had the highest activity of the antineoplastic agents tested; that of mitomycin C was low and nitromin, 6-diazo-5-oxonorleucine (6-DON), and azaserine were inactive.

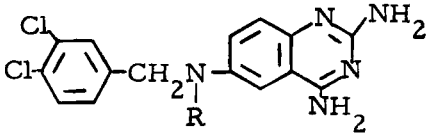
Among 64 derivatives of 2-chloro-1, 4-naphthoquinone[19] two (III and IV) showed high activity:



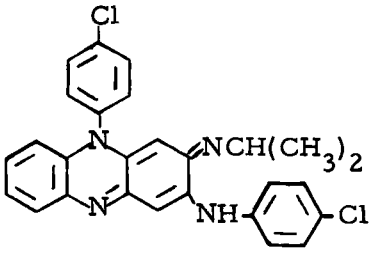
III.



IV.



V. R = -H (PAM-1392)
VI. R = -NO (CI-679)



VII. B663

Two triaminoquinazoline[20] compounds have been found active: 2, 4-diamino-6-(3, 4-dichlorobenzylamino) quinazoline (V) and 2, 4-diamino-6(3, 4-dichlorobenzyl) nitrosoaminoquinazoline (VI). The first (PAM-1392) was several times more active than quinine, rapid, synergistic with sulfadiazine and effective against strains highly resistant to chloroquine, DDS, chloroguanyl HCl or pyrimethamine but had no appreciable repository activity in mice. The second (CI-679) was about 500-fold more potent than

quinine, rapid, synergistic with DDS and also effective against the resistant strains, having repository activity for several weeks in mice.

A phenazine compound[21] known as B663 with antituberculosis activity was found to be suppressive against both chloroquine-sensitive and resistant strains. Testing of additional members of the series has been suggested.

All of the above compounds were active against P. berghei. Against P. vinckei in mice, cyclophosphamide and actinomycin D were more effective than chloroquine[22]. P. chabaudi in mice has also been reinvestigated as a system for use in antimalarial screening with observations on metachloridine and proguanil being new[23].

A new and interesting approach to experimental therapy of malaria has been the use of interferon inducers, which act not on the parasite but on the host, to stimulate the production of an endogenous inhibitor of parasite reproduction. Three different types of interferon inducers have been shown to be capable of affecting the course of mouse malaria: New Castle Disease Virus (NDV), statolon, and the complex of polyriboinosinic and polyribocytidylic acids know as rI:rC[24]. These three materials have in common ribonucleic acid, the rI:rC copolymer consisting of a double-stranded chain of ribonucleotides, the active component of statolon containing a double-stranded RNA of presumably viral origin, and NDV containing a single stranded RNA which usually replicates poorly in mice and it is not known whether a double-stranded replicative intermediate of NDV-RNA is formed. Interferon studies have stirred the imagination of microbiologists and immunologists and the possibility that a variety of polymeric substances other than those already demonstrated to be active may have similar properties presents a chemical and biochemical challenge.

Avian malaria - Bird malaria was long used as a primary screening system before the introduction of rodent malaria to chemotherapeutic investigation in 1949. Now it is used comparatively rarely as a screen and is more likely to be found in specialized studies such as those on the effects of chloroquine on the morphology of the erythrocytic stages of P. gallinaceum[25] or on the effects of selected drugs on the oocysts or sporozoites of P. gallinaceum in mosquitoes[26].

Simian malaria - Monkeys with Plasmodium cynomolgi or P. knowlesi are often used in the preclinical evaluation of potential antimalarials as well as being employed in the elucidation of various aspects of action of compounds which are already in clinical use. Examples of compounds which have reached the simian phase of preclinical evaluation are the chlorinated lincomycin derivatives[27,28] and the triaminoquinazolines[20].

Although lincomycin itself is essentially inactive as an antimalarial 1'-demethyl-7(S)-chloroanalogs have good antimalarial activity, several being

comparable to chloroquine and DDS in P. berghei infections. Three of these were tested against P. cynomolgi in rhesus monkeys and found to be active although they were slow in clearing parasites from the blood requiring 3 to 6 days after the 5 day treatment period. In view of their activity against strains of P. berghei resistant to chloroquine, pyrimethamine, primaquine quinine and DDS their trial in clinical malaria has been suggested.

The quinazoline PAM-1392 (V), when given appropriately, cured infections in rhesus monkeys with P. cynomolgi or P. knowlesi. The quinazoline CI-679 (VI) was highly potent and curative in P. cynomolgi infections and when given parenterally was prophylactic for several months[20].

Examples of the use of simian malaria in retrospective studies of antimalarials in clinical use are the investigations on the action of chloroquine on P. knowlesi[29,30] and those on trimethorprim alone[31] against P. cynomolgi or in combination with sulfalene against P. knowlesi[32].

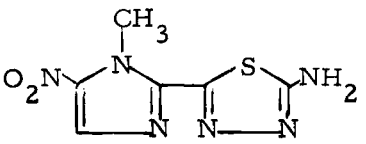
Human malaria - The reports of individual studies using well-known compounds are numerous. There are some interesting reviews[33-35].

Drug resistance in malaria - Although this problem lurks in the background of all chemotherapeutic investigations it is a field of specialization which continues to be studied in detail[36-44] and reviewed by experts[45,46].

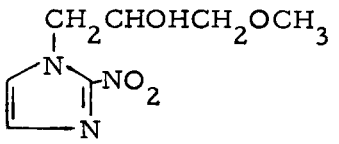
OTHER HUMAN PROTOZOAL INFECTIONS

Amebiasis - Studies on the synthesis of emetine and its relatives[47,48] continue and evidence is presented suggesting that the activity and toxicity of racemic 2-dehydroemetine are attributable principally to the levo enantiomer, while the dextro form is relatively inactive. In vitro and in amebic liver abscess of the hamster no difference in activity could be detected between natural levo emetine, racemic 2-dehydroemetine and levo-2-dehydroemetine[49]. Against rat caecal infections natural levo emetine and levo 2-dehydroemetine had similar activities, about twice that of racemic 2-dehydroemetine. The more active compounds were also about twice as toxic.

Certain nitro derivatives of arylsydnones[50] have high in vitro activity against E. histolytica, equivalent to that of entobex.



VIII. CL 75,805



IX. RO 7-0582

The compound CL 75,805 (VIII) is reported to have activity against both intestinal amebiasis in rats and hepatic amebiasis in hamsters, more potent than the other amebicides in general, except for emetine[51]. From a series of 2-nitroimidazoles, non-specifically noted last year, one (IX) has been selected for extensive evaluation[52].

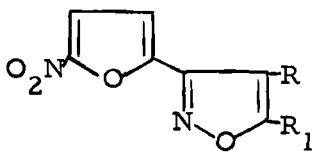
Certain amino-acridines, benz [c] acridines and benzo [b][1,5] naphthyridines[53], which were also antimalarial, were active against E. histolytica in vitro and in rats. In a series of thirty four 2-amino-5 nitrothiazoles[54] good in vitro activity against E. histolytica was found in 5 but it was somewhat less than that in emetine and paromomycin.

Current therapeutic practice in amebiasis has been reviewed[55].

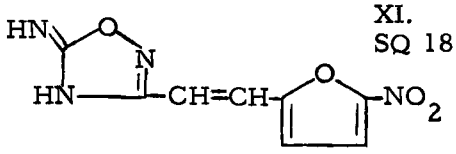
Balantidiasis - The activity of metronidazole[56] in vitro against Balantidium coli of both human and porcine origin suggests its possible usefulness in this disease. Oxytetracycline is currently used clinically[57]

Leishmaniasis - During studies on experimental amyloidosis it was noted that gold sodium thiomalate influenced the course of leishmaniasis in the hamster and a detailed study[58] showed that it completely supressed growth of L. donovani in 13 of 15 hamsters and that it had in vitro activity equivalent to that of Amphotericin B.

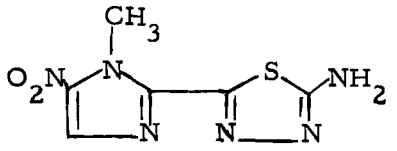
X.



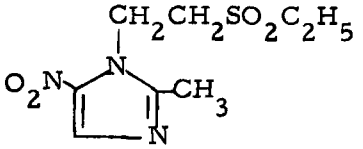
XI.
SQ 18,506



XII.



XIII.



Trichomoniasis - Although no clinical reports on new antitrichomonal compounds have been noted, a number of nitrofurans and nitroimidazoles have been under study experimentally. There is a large series of 3- or 5-(5-nitro-2-furyl)-5-or-3-methylisoxazoles[59] (X) as well as XI (SQ 18,506) and its relatives[60-62], a thiadiazole[63] (XII) and XIII[64-66].

In a series of thirty four 2-amino-5-nitrothiazoles[54] good in vitro antitrichomonal activity was found in 12 but in vivo against T. vaginalis in mice none was more than 1/4 as potent as metronidazole.

Trypanosomiasis - Although there are no reports of clinical trials of new preparations for trypanosomiasis there are a few compounds in the experimental stage. Compound XII

effected cures in mice in acute Trypanosoma equiperdum infections and subacute T. cruzi infections by both oral and parenteral routes[67]. The activity of certain nitrofurans related to nitrofurantion against T. congolese, T. cruzi, T. gambiese, and T. rhodesiense is not unexpected[68].

ANIMAL PROTOZOAL INFECTIONS

Aegyptianellosis - There has been no satisfactory treatment until recently[69] for infections with the blood parasite of chickens, Aegyptianella pullorum, which has been shown to be sensitive to tetracycline, chlortetracycline, doxocycline, and oxytetracycline given either prophylactically or therapeutically per os[70].

Coccidiosis - This field of poultry disease, caused by a number of species of the genus Eimeria has continued to be highly competitive and the recurrent emergence of resistant strains has inspired new synthesis and mixtures of compounds. Representatives of three different types are the trifluoromethylbenzanilides[71], ethyl 6, 7-bis (cyclopropylmethoxy)-4-hydroxy-3-quinolinecarboxylate[72], and a mixture of N'-(2, 6-dimethoxy-4-pyrimidinyl) sulfanilamide and 2, 4-diamino-5-(4, 5-dimethoxy-2-methylbenzyl pyrimidine[73,74]. The first group has been tested against E. tenella only but the two latter types have shown broad activity against a number of other species as well: E. acervulina, brunetti, maxima and necatrix.

Studies on the acridines have shown that acriflavine can mediate a reversion to sensitivity in an amprolium resistant strain[75,76] but that quinacrine does not prevent development of resistance to glycarbylamide[77].

Histomoniasis - In a series of substituted benzoic acid (5-nitrofurfurylidene hydrazides[78] one showed outstanding activity against Histomonas meleagridis in turkeys and chickens: 3, 5-dinitrosalicylic acid (5-nitrofurfurylidene) hydrazide.

In a series of 1, 2-disubstituted 5-nitroimidazoles[79] the most potent was 1-methyl-2-isopropyl-5 nitroimidazole which was 4 to 8 times more effective than others in the series and twice as active as dimetridazole in turkeys. Compound XIII is also reported to be active in histomoniasis in chickens[64,65].

Trichomoniasis - Although none of the new antitrichomonal compounds reported from screening against either T. vaginalis or T. foetus in experimental animals[54,59-66] has yet reached trial in large animals, dimetridazole, previously used orally against Trichomonus foetus in bulls has been shown to be effective on single intravenous administration which effects great saving in time and material[80]. Intestinal trichomoniasis in horses, which has been reported more frequently recently, is said to be

effectively controlled by the use of iodochlorhydroxyquin in the drinking water[81].

HUMAN HELMINTH INFECTIONS

Reviews, conferences, symposia - There are some general reviews[82,83] of current therapy as well as a discussion of some biochemical effects of anthelminthic drugs[84]. Individual drugs considered at specific meetings were niridazole[85] and thiabendazole[86].

Schistosomiasis - Mouse infections with Schistosoma mansoni or S. japonicum continue to be used in primary screening with interesting compounds being subsequently evaluated in hamsters, and monkeys usually being the final preclinical experimental animal.

In a series of compounds of structure II three were active against S. mansoni in mice[15].

The effect of hydroxylation on activity of xanthenones and 4-methyl-3-chloroanilines was studied with S. mansoni in both mice and hamsters and was found to enhance schistosomicidal activity one- to six-fold in the mouse and two- to 33-fold in the hamster[87].

A new series of 2-aminomethyltetrahydroquinoline derivatives[88] had activity against S. mansoni in mice. Metabolic studies showed that several species of animals hydroxylated the 6-methyl group. One of these metabolites, 6-hydroxymethyl-2 isopropylaminomethyl-7-nitro-1, 2, 3, 4-tetrahydroquinoline has been shown to be very active against S. mansoni in vervet monkeys (Cercopithecus aethiops), especially by parenteral administration, and the series is being investigated further.

A new nitrofuran, (XI) (SQ 18,506) was active in mice against both S. mansoni and S. japonicum[89].

Tapeworm - The disappearance of Hymenolepis nana eggs from the feces of a child during a 7 days treatment with emetine HCl suggests an extension of the spectrum of this established compound[90]. Some 9-aminoacridines and a related benz [c] acridine had activity against H. nana in mice equivalent or superior to that of quinacrine[53]. Three out of 10 arylsydnones, which were also quite active against E. histolytica in vitro, had activity against H. nana in mice, a little inferior to that of quinacrine[50].

Angiostrongylosis - Levo-tetramisole has been shown to effect reductions in numbers of migrating larvae of Angiostrongylus cantonensis in rats[91] but neither it nor thiabendazole, the only other drug shown to be active in this infection, is reported to have been tested in man.

Ascariasis - Levo-tetramisole has also been shown to be more potent than the racemic form in Ascaris lumbricoides infections[92].

Capillariasis - Thiabendazole has been reported as the drug of choice for infections with Capillaria philippinensis, the epidemic intestinal form which causes enteropathy and malabsorption[93]. Capillaria hepatica, the liver parasite of rats and numerous other mammals and occasionally man, is said to be susceptible to methyridine[94].

Enterobiasis - Activity against pinworms in mice has been reported for certain series of compounds which have not reached clinical trial. Some derivatives of 9-aminoacridine and related benz [c] acridine and benzo [b] [1, 5] naphthyridine[53] had activity against Syphacia obvelata as Aspiculuris tetraptera in mice. Three of 10 arylsydnones[50] had activity against S. obvelata, somewhat lower than that of piperazine.

Filariasis - Animal models, notably Litomosoides carinii and Brugia pahangi in cotton rats and Dipetalonema witei in gerbils, continue in use for screening of possible antifilarial agents, and certain nitrofurans[68] related to nitrofurantoin have been found to have some activity in these infections. A tissue culture system in which microfilariae of the human parasite Wuchereria bancrofti can be maintained in an actively motile state from 24 to 48 hours has recently been devised and it is hoped this may provide a useful method for the study of drugs which act against larval stage[95].

Hookworm - Although no new compounds have been reported in the clinic, a series of imidoylureas has been synthesized and tested against the dog hookworms Ancylostoma caninum and Uncinaria stenocephala. The most active was 1-(p-chlorophenyl)-3-pentanimidoylurea, which was well tolerated and is being examined further[96]. Of passing interest may be the effect of ingested garlic on the larvae of Necator americanus in the feces[97]. The levo isomer of tetramisole has been found to be more active than the racemic compound in hookworm infections of man[92].

Trichinosis - Although some studies fail to show that thiabendazole has any definite effect on the course of Trichinella spiralis infections in man[98] it is considered by some to be the drug of choice in this disease where the therapeutic agents should be active against the larvae[99]. In mice parbendazole, methyl-5(6)-butyl-2-benzimidazolecarbamate, effects great reductions in the number of larvae in the tissues when given in the diet for 7 days, 1 to 12 weeks after infection[100].

ANIMAL HELMINTH INFECTIONS

Review - A 20 page article covers activity against nematodes, tapeworms and flukes of various compounds mentioned in previous reports[101].

Fascioliasis - Comparative studies on the more frequently used preparations have been made[102-104] and the importance of age and size of infection has been emphasized[105]. Emetine is reported to be effective against the immature forms of Fasciola hepatica in the liver tissue in both rats and sheep[106]. Synergism has been demonstrated between hexachlorophane and 4-cyano-2-iodo-6-nitrophenol in sheep[107].

A new agent reported to have activity in both sheep and cattle and against immature as well as adult flukes is 4, 4', 6, 6'-tetrabromo-2, 2'-biphenyldiol mono (dihydrogen phosphate)[108]. Another is 3, 5-diiodo-3'-chloro-4'-(p-chlorophenoxy)-salicylanilide which is also active against immature flukes in sheep[109,110].

Nematodes - Notable evaluations have been made of the efficacy of parbendazole (SKF 29044), methyl 5(6)-butyl-2-benzimidazolecarbamate, in cattle[111,112], goats[113], sheep[114] and swine[115]. Levo-tetramisole has been similarly studied in cattle[116-119] and sheep[120], and both of the above have been compared with thiabendazole in sheep[121]. New compounds with activity against gastrointestinal nematodes in sheep are aminopentadienylideneammonium salts in which the N atoms are part of a piperidine of pyrrolidine ring[122] and 3, 5-diiodo-3'-chloro-4'-(p-chlorophenoxy)-salicylanilide[123] which has outstanding activity against Haemonchus contortus.

References

1. D. M. Aviado, Exp. Parasit., 25, 399 (1969).
2. C. Sadavongvivad and D. M. Aviado, ibid, 24, 313 (1969).
3. M. A. Silver and D. M. Aviado, ibid, 24, 152 (1969).
4. D. M. Aviado, Y. W. Cho and J. M. Smith, ibid, 25, 283 (1969).
5. D. M. Aviado and H. A. Reutter, Jr., ibid, 26, 314 (1969).
6. D. M. Aviado and P. J. Cambar, ibid, 26, 354 (1969).
7. D. M. Bailey, J. Med. Chem., 12, 184 (1969).
8. T. Singh, R. G. Stein and J. H. Biel, ibid, 949 (1969).
9. T. Singh, R. G. Stein and J. H. Biel, ibid, 801 (1969).
10. T. Singh, R. G. Stein and J. H. Biel, ibid, 368 (1969).
11. E. F. Elslager, Z. B. Gavrilis, A. A. Phillips and D. F. Worth, ibid, 357 (1969).
12. D. F. Worth, E. F. Elslager and A. A. Phillips, ibid, 591 (1969).
13. E. F. Elslager, D. B. Capps and D. F. Worth, ibid, 597 (1969).
14. E. F. Elslager, A. A. Phillips and D. F. Worth, ibid, 363 (1969).
15. E. F. Elslager and A. A. Phillips, ibid, 519 (1969).
16. H. Bader, J. F. Hoops, J. H. Biel, H. H. Koelling, R. G. Stein and T. Singh, ibid, 709 (1969).
17. B. Serafin, Y. Urbanski and D. C. Warhurst, ibid, 336 (1969).
18. P. M. L. Siu, Proc. Soc. Exp. Biol. Med., 129, 753 (1968).

19. B. Prescott, J. Med. Chem., 12, 181 (1969).
20. P. E. Thompson, A. Bayles and B. Olszewski, Abstracts of Papers, 44th Ann. Meeting Amer. Soc. Parasitologists, Washington, D. C. November 3-7, 1969.
21. J. N. Sheagren, J. Parasit., 54, 1250 (1968).
22. E. Fink and D. M. Goldenberg, Proc. Soc. Exp. Biol. Med., 132, 165 (1969).
23. K. J. Ott, Exp. Parasit., 24, 194 (1969).
24. R. I. Jahiel, R. S. Nussenzweig, J. Vilcek and J. Vanderberg, Amer. J. Trop. Med. Hyg., 18, 823 (1969).
25. M. Aikawa and R. L. Beaudoin, ibid, 166 (1969).
26. E. J. Gerberg, L. T. Richard and J. B. Poole, Mosquito News, 26, 359 (1966).
27. K. G. Powers, Amer. J. Trop. Med. Hyg., 18, 485 (1969).
28. B. J. Magerlein and F. Kagan, J. Med. Chem., 12, 780 (1969).
29. S. N. Ali, K. A. Fletcher and B. G. Maegraith, Trans. Roy. Soc. Trop. Med. Hyg., 63, 3 (1969).
30. D. C. Warhurst, ibid, 4 (1969).
31. L. H. Schmidt, J. Harrison, R. Ellison and P. Worcester, Proc. Soc. Exp. Biol. Med., 131, 294 (1969).
32. W. E. Rothe, D. P. Jacobus and W. G. Walter, Amer. J. Trop. Med. Hyg., 18, 491 (1969).
33. D. A. Berberian, Amer. J. Med., 46, 96 (1969).
34. R. D. Powell, "Current Therapy", W. B. Saunders Co., Philadelphia, 1969, pp. 23-26, Ed. H. F. Conn.
35. L. G. Hunsicker, Arch. Int. Med., 123, 645 (1969).
36 W. Peters, Ann. Trop. Med. Parasit., 62, 238 (1968).
37. W. Peters, ibid, 246 (1968).
38. R. E. Howells, W. Peters and E. A. Thomas, ibid, 267 (1968).
39. R. E. Howells, W. Peters and E. A. Thomas, ibid, 271 (1968).
40. W. Peters, ibid, 277 (1968).
41. W. N. Beesley and W. Peters, ibid, 288 (1968).
42. A. E. Ramkaran and W. Peters, Nature, 223, 635 (1969).
43. W. Peters, ibid, 858 (1969).
44. M. Yoeli, R. S. Upmanis and H. Most, Parasit., 59, 429 (1969).
45. L. H. Schmidt, Ann. Rev. Microbiol., 23, 427 (1969).
46. W. Peters, Trans. Roy. Soc. Trop. Med. Hyg., 63, 25 (1969).
47. N. Whittaker, J. Chem. Soc. (Org.), 1, 94 (1969).
48. H. T. Openshaw, N. C. Robson and N. Whittaker, ibid, 105 (1969).
49. P. Johnson and R. A. Neal, Ann. Trop. Med. Parasit., 62, 455 (1968).
50. G. Pala, A. Mantegani, G. Coppi and R. Genova, Bull. de Chimie Therapeutique, 1, 31 (1969).
51. S. Carvajal, S. Padacks, H. Steinmann, H. Maxon and A. Wozniak, Antimicrobial Agents and Chemotherapy, 1968, pp. 541-544.

52. H. N. Prince, E. Grunberg, E. Titsworth and W. F. DeLorenzo, Appl. Microbiol., 18, 728 (1969).
53. E. F. Elslager and D. F. Worth, J. Med. Chem., 12, 955 (1969).
54. L. M. Werbel, E. F. Elslager, A. A. Phillips, D. F. Worth, P. J. Islip and M. C. Neville, ibid, 521 (1969).
55. S. F. Powell, "Current Therapy", W. B. Saunders Co., Philadelphia, 1969, pp. 3-6, Ed. H. F. Conn.
56. V. Zaman and P. N. Natarajan, Trans. Roy. Soc. Trop. Med. Hyg., 63, 152 (1969).
57. M. T. Hoekenga, "Current Therapy", W. B. Saunders Co., Philadelphia, 1969, pp. 33-35, Ed. H. F. Conn.
58. J. L. Abruzzo and R. J. DeHoratius, Amer. J. Trop. Med. Hyg., 18, 506 (1969).
59. R. G. Micetich, J. Med. Chem., 12, 611 (1969).
60. H. Brever and H. Basch, Abstracts of Papers, Ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D. C., October 27-29, 1969, p. 41.
61. H. Basch, H. Gadebusch and R. Semar, ibid, p. 41-42.
62. R. Semar and H. Gadebusch, ibid, p. 41-42.
63. E. J. Burden, E. Schumacher and M. Kelley, Antimicrobial Agents and Chemotherapy, 1968, pp. 538-540.
64. M. W. Miller, H. L. Howes, R. V. Kasubick and A. R. English, Abstracts of Papers, Ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D. C., October 27-29, 1969, p. 32.
65. H. L. Howes, J. E. Lynch and J. L. Kivlin, ibid, p. 32-33.
66. J. A. Taylor, Jr., J. R. Migliardi and M. S. vonWittenau, ibid, p. 33.
67. E. J. Burden and E. Racette, Antimicrobial Agents and Chemotherapy, 1968, p. 545-547.
68. R. Foster, G. Pringle, D. F. King and J. Paris, Ann. Trop. Med. Parasit., 63, 95 (1969).
69. G. Lämmler and R. Gothe, Zeit. für Tropenmed. Parasit., 18, 479 (1967).
70. G. Lämmler and R. Gothe, Zbl. Vet. Med., B, 16, 663 (1969).
71. D. E. Welch and R. R. Baron, J. Med. Chem., 12, 957 (1969).
72. R. H. Mizzoni, F. Goble, J. Szanto, D. C. Maplesden, J. E. Brown, J. Boxer and G. deStevens, Experientia, 24, 1188 (1968).
73. M. Mitrovic, E. G. Schildknecht and G. Fusiek, Poul. Sci., 48, 210 (1969).
74. W. L. Marusich, E. OGrinz, M. Brand and M. Mitrovic, ibid, 217 (1969).
75. D. K. McLoughlin and M. B. Chute, J. Parasit., 54, 582 (1968).
76. D. K. McLoughlin and M. B. Chute, ibid, 55, 660 (1969).
77. D. K. McLoughlin and J. L. Gardiner, ibid, 55, 208 (1969).
78. E. W. Berndt, H. VanEssen and B. G. Held, J. Med. Chem., 12, 371 (1969).
79. M. Mitrovie, M. Hoffer and E. G. Schildknecht, Antimicrobial Agents and Chemotherapy, 1968, p. 445-448.
80. D. K. McLoughlin, J. Parasit., 54, 1038 (1968).
81. S. P. Bennett and D. A. Franco, J. Amer. Vet. Med. Ass., 154, 58 (1969).

82. H. W. Brown, Clinical Pharmacol. Ther., 10, 5 (1969).
83. H. M. Gilles, Practitioner, 203, 518 (1969).
84. E. Bueding, Biochem. Pharmacol., 18, 1541 (1969).
85. F. C. Goble, editor, Ann. N. Y. Acad. Science, 160 (2), 423-946 (1969).
86. O. J. Stone, editor, Texas Reports Biol. Med., 27 (Suppl. 2), 533-708 (1969).
87. D. A. Berberian, E. D. Dennis and H. Freele, J. Med. Chem., 12, 607 (1969).
88. H. C. Richards and R. Foster, Nature, 222, 581 (1969).
89. E. Bueding and C. H. Robinson, Abstracts of Papers, Ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D. C., October 27-29, 1969, p. 42.
90. R. D. P. Eaton, Trans. Roy. Soc. Trop. Med. Hyg., 63, 153 (1969).
91. K. Jindrak and J. E. Alicata, Chemotherapy, 14, 244 (1969).
92. D. Thienpont, J. Brugmans and K. Abadi, Amer. J. Trop. Med. Hyg., 18, 520 (1969).
93. G. E. Whalen, G. T. Strickland, H. H. Cross, C. Uylangco, E. B. Rosenberg, R. A. Gutman, R. H. Watten and J. J. Dizon, Lancet, 1, 13 (1969).
94. A. H. Waddell, Ann. Trop. Med. Parasit., 63, 63 (1969).
95. J. K. Sarkar, Trans. Roy. Soc. Trop. Med. Hyg., 63, 374 (1969).
96. G. D. Diana, A. Yarinsky, E. S. Zalay, D. A. Berberian and S. Schalit, J. Med. Chem., 12, 791 (1969).
97. G. J. Bastidas, Amer. J. Trop. Med. Hyg., 18, 920 (1969).
98. Y. Thibaudeau and J. J. Gagnon, Canad. Med. Ass. J., 101, 533 (1969).
99. J. P. Sanford, "Current Therapy", W. B. Saunders Co., Philadelphia, 1969, p. 79-80, Ed. H. F. Conn.
100. V. J. Theodorides and M. Laderman, J. Parasit., 55, 678 (1969).
101. T. E. Gibson, Advances in Parasitology, 7, 349 (1969).
102. J. C. Boray and F. Happich, Aust. Vet. J., 44, 72 (1968).
103. J. C. Boray, F. A. Happich and W. O. Jones, ibid, 45, 94 (1969).
104. K. B. Sinclair, Vet. Rec., 84, 544 (1969).
105. E. Thorpe, Ann. Trop. Med. Parasit., 62, 361 (1968).
106. M. S. Grant and S. E. Jaggers, Res. Vet. Sci., 10, 197 (1969).
107. S. B. Kendall and M. A. Peirce, Brit. Vet. J., 125, 82 (1969).
108. S. van der Meer and H. Powwels, J. Med. Chem., 12, 534 (1969).
109. H. Mrozik, H. Jones, J. Friedman, G. Schwartzkipf, R. A. Schardt, A. A. Patchett, D. R. Hoff, J. J. Yakstis, R. F. Riek, D. A. Ostland, G. A. Plishker, R. W. Butler, A. C. Cuckler and W. C. Campbell, Experientia, 25, 883 (1969).
110. W. C. Campbell, D. A. Ostland, R. F. Riek and J. J. Yakstis, Abstracts of Papers, 44th Annual Meeting Amer. Soc. Parasitologists, Washington, D. C., November 3-7, 1969.
111. R. Rubin, Amer. J. Vet. Res., 29, 1385 (1968).
112. R. Rubin, J. Amer. Vet. Med. Assoc., 154, 177 (1969).

113. V. J. Theodorides, G. C. Scott and M. Laderman, Amer. J. Vet. Res., 30, 1887 (1969).
114. G. Lämmler, B. N. Sahai and H. Zahner, Brit. Vet. J., 125, 205 (1969).
115. J. Chang and R. B. Wescott, Amer. J. Vet. Res., 30, 77 (1969).
116. J. E. Alicata and H. H. Furumoto, ibid, 139 (1969).
117. H. Ciordia and D. M. Baird, ibid, 1145 (1969).
118. J. A. Turton, Vet. Rec., 85, 264 (1969).
119. G. H. Rohrbacher, J. Emro and E. Waletzky, Abstracts of Papers, 44th Annual Meeting Amer. Soc. Parasitologists, Washington, D. C., November 3-7, 1969.
120. J. A. Hart, P. S. James and C. Curr, Aust. Vet. J., 45, 73 (1969).
121. K. C. Kates, M. L. Colglazier, F. D. Enzie, I. L. Lindahl and G. Samuelson, Jr., ibid.
122. D. L. Garmaise, G. Y. Paris, J. Komlossy and R. C. McCrae, J. Med. Chem., 12, 806 (1969).
123. J. R. Egerton and W. C. Campbell, Abstracts of Papers, 44th Annual Meeting Amer. Soc. Parasitologists, Washington, D. C., November 3-7, 1969.

Chapter 13. Antifungal Agents

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The appearance in debilitated patients of opportunistic fungal infections after therapy with antibiotics and corticosteroids or other immunosuppressive agents continues to be emphasized.[1-3] Since these medicaments are vital in procedures such as the transplantation of organs, antifungal agents become more important for prophylactic or therapeutic use.[4] All indications are that they will continue to increase in importance.

Clnical Experience - More than 10 years' experience has shown amphotericin B to be the most effective drug against many of the deep mycoses.[5,6] A number of excellent reviews have examined treatment schedules with a view to minimizing toxic reactions. In cryptococcosis, the mere finding of *Cryptococcus neoformans* in clinical specimens does not justify the use of amphotericin B. In the pulmonary form of cryptococcosis, use of amphotericin B is apparently not indicated after unilateral resection of the lung, but the drug should be administered for diffuse bilateral disease or for extension of a localized lesion.[7] In cryptococcal meningitis, daily intrathecal administration of 0.3 mg of amphotericin B as a 1-hour infusion gave optimal cerebrospinal fluid levels with minimal toxic symptoms.[8,9]

Amphotericin B appears to be the most effective drug in rapidly advancing blastomycosis.[10] A review study of 93 patients with blastomycosis showed a significantly greater relapse rate among patients who received less than a total of 1.5 g of amphotericin B, or 20 mg/kg of body weight per day.

A review of the treatment of pulmonary histoplasmosis suggests that a dosage of 50 mg/day, three times per week, for a total dose of 2-3 g over a 3 to 4-month period is the best therapy for the progressive cavitary form of the disease. After resection of the lung, a course of 2 g will prevent recurrence.[11] It has been suggested that prolonged administration of the drug at sub-optimal levels is an effective method for treating both histoplasmosis and paracoccidioidomycosis.[12]

A review of the therapy of systemic *Candida albicans*

infections emphasized that by judicious administration of amphotericin B with careful monitoring of the patient, toxic side effects can be so minimized that the drug may be administered safely even to infants and to patients with high levels of blood-urea-nitrogen.[13]

Amphotericin B continues to be effective and necessary in pulmonary sporotrichosis, a condition perhaps more common than previously recognized.[14] Iodides, apparently effective in the cutaneous form of the disease, are not effective in the pulmonary form.[15] Amphotericin B has been reported to be effective in several rare disease forms, such as blastomycosis of the bone, where intramedullary and intravenous administration effected an apparent cure;[16] maduromycosis caused by Monosporium apiospermum, where infusion into the lesion produced a transitory improvement;[17] orbital histomplasmosis caused by Histoplasma duboisii, where the lung was employed pre- and post-surgically;[18] and histoplasmal endocarditis.[19] Of special interest was the successful treatment of allergic bronchopulmonary aspergillosis by aerosol nebulization of amphotericin B plus sodium iodide.[20]

Concomitant prophylactic administration of amphotericin B with tetracycline antibiotics continues to be studied. Several reports enthusiastically defend the combination as preventing candidal overgrowth, or even eliminating the organism,[21,22] whereas another report casts doubt on the utility of the procedure.[23]

Hamycin was reported to be more active in vitro against 77 strains of C. neoformans than was amphotericin B.[24] However, when three strains were tested in mice, amphotericin B was more effective.[25]

Candicidin, in the form of an ointment or vaginal suppositories, gave rapid symptomatic relief and produced negative cultures in 23 of 24 cases of Candida vaginitis.[26]

The oral toxicity of levorin A, a recently reported heptaene macrolide, was studied in laboratory animals. A dose of 30 mg/kg per day appeared to be safe.[27] A soluble sodium salt was employed with considerable success as an aerosol for inhalation therapy of patients with Candida infection of the lungs and bronchi.[28]

Neoheptaene, another recently reported heptaene macrolide, when administered intraperitoneally was active in experimental murine histoplasmosis, blastomycosis, and cryptococcosis, but not in coccidioidomycosis. The dosage required approached or overlapped the toxic range.[29]

Nystatin was effective in single cases of pulmonary candidosis and pulmonary aspergillosis when administered by aerosol inhalation.[30,31] An interesting finding was the detection of measurable blood levels in the latter case.

Pimaricin in ointment form was effective in cutaneous Candida infections, although relapse was common in patients with paronychia. Vaginal suppositories appeared effective in Candida vulvo-vaginitis.[32] A 5% pimaricin suspension containing potassium iodide gave good results in experimental keratomycosis in rabbits, with both C. albicans and Aspergillus fumigatus as the infecting organisms.[33]

Griseofulvin continues to be a mainstay in the oral treatment of fungal skin diseases. Oral administration of griseofulvin has reduced hospitalization time for American troops in Vietnam, where dermatophytoses caused by Trichophyton mentagrophytes are predominant. The arduous conditions of tropical campaigning make topical medicaments almost useless.[34] Several reports on toxic side effects have appeared. When griseofulvin was administered to pregnant rats at 50 to 500 mg/kg, there was a teratogenic effect on the fetuses proportional to the dose. It was suggested that griseofulvin not be given to pregnant women.[35] Griseofulvin, administered orally to a young girl, produced hypertrophy of the mammary glands and bleeding from the genitalia.[36]

Pyrrolnitrin was investigated in mice for its activity against systemic mycotic infections.[37] The drug was administered subcutaneously in multiple doses. Some activity was observed in candidosis and cryptococcosis, but not in blastomycosis and histoplasmosis. Three new bromo analogs of pyrrolnitrin were detected in fermentations of Pseudomonas pyrrolnitrica when the medium was supplemented with NH_4Br. They were less active than pyrrolnitrin in vitro.[38]

Saramycetin (X5079C; Ro2-7758) has long been in such short supply as to prevent adequate clinical trials. An additional supply has now been produced[39] by a much more

economical method.[40] A study of the comparative effectiveness of saramycetin and amphotericin B in the treatment of cavitary pulmonary histoplasmosis is now underway.[41]

The *in vitro* activity of mycophenolic acid against *C. albicans*, *C. neoformans* and several *Trichophyton* sp. was measured. The antibiotic had some effect on experimental infections with *T. asteroides*, but failed to cure them, indicating a fungistatic rather than fungicidal action.[42]

Chlorodantoin [5-(1-ethylamyl)-3-trichloromethylthiohydantoin] in the form of a 1% cream has shown considerable activity in *Candida* vaginitis.[43]

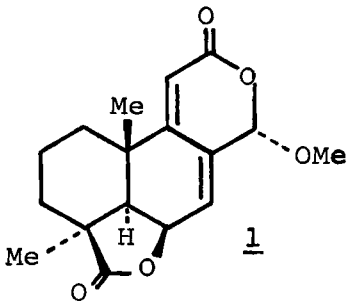
5-Fluorocytosine was active in prolonging the life of mice infected with *C. albicans* when administered intraperitoneally in single doses of 87.5 to 200 mg/kg. On the basis of differential *in vitro* sensitivities, it was suggested that deamination of the drug to 5-fluorouracil does not occur.[44] A single patient with cryptococcal meningitis responded to treatment with 100-200 mg/kg daily.[45]

Several reviews of the treatment of blastomycosis discuss the relative efficacies of 2-hydroxystilbamidine isothionate (2-HI) and amphotericin B in a large number of cases.[10,46] In general, it appears that amphotericin B, because of its toxic side effects, should be reserved for use in rapidly advancing disease, especially if the central nervous system is involved, or for treating cases that have not responded to 2-HI. 2-HI should be used in cases of slowly progressive disease or those having only skin lesions.

Fentichlor (2,2'-dihydroxy-5,5'-dichlorodiphenyl sulfide), a German drug little known in the U.S., caused clinical improvement in a case of maduromycosis due to *M. apiospermum* when infused locally at a concentration of 1%. It is suggested as safe for use in selected infections.[17]

New Antifungal Agents - The structure of LL-Z1271α a terpenoid metabolite from an *Acrostalagmus* species, has been determined to be *1*. It possesses *in vivo* activity against ringworm infections in guinea pigs.[47] A structurally related antibiotic, siccanin, has been reported to be effective topically against *T. mentagrophytes* infections in guinea pigs.[48] Its structure, *2*, has been determined by x-ray

crystallography. [49]



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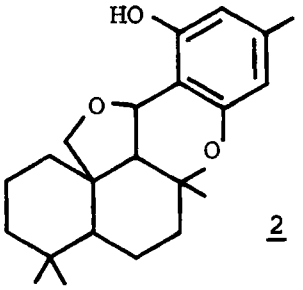
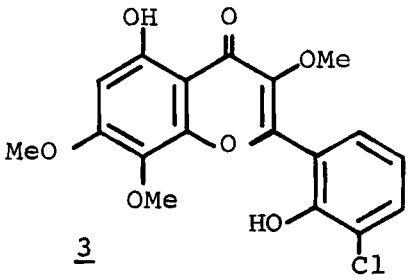


2

A fungal metabolite, chlorflavonin, with highly specific *in vitro* activity against a few species of fungi, has structure 3, and is reported to be the first fully characterized flavone fungal metabolite and the first naturally occurring chlorinated flavone.[50,51]



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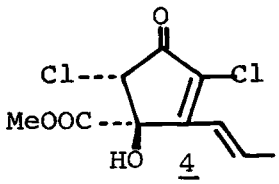
The structures of three chlorinated fungitoxic fungal metabolites, 4, 5, and 6, have been determined by a combination of x-ray and spectral studies.[52] Cryptosporiopsin[53] was shown to be identical to 4.



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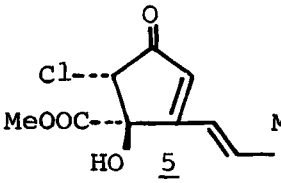

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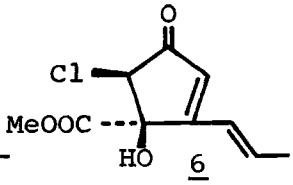
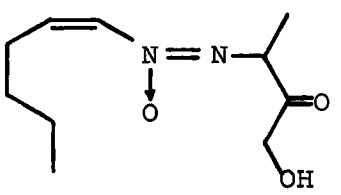


6

An antibiotic, LL-BH872α, structurally related to elaiomycin, has been shown[54] to be 7. Structure 8 has been assigned for versicolin.[55] A derivative, 9, of alkannin that is active against *C. albicans* and *C. neoformans in vitro* was isolated from the roots of *Arnebia nobilis*.[56]



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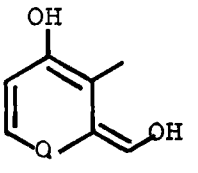

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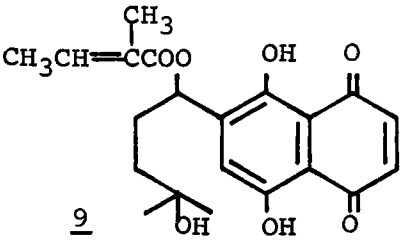
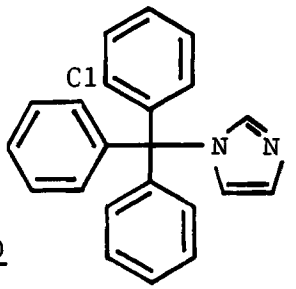


9

Several other new antibiotics showing in vitro antifungal activity have been isolated and partially characterized chemically: oryzachlorin ($C_{26}H_{31}O_8N_2S_2Cl$), active against yeasts;[57] gonidodomin ($C_{43}H_{58}O_{11}$), isolated from a marine dinoflagellate and inhibiting Cryptococcus and Trichophyton, among others;[58] subsporins A,B, and C (peptide);[59] hondamycin ($C_{47}H_{78}O_{13}$);[60] aabomycin A ($C_{39-40}H_{65-67}O_{11}N$);[61] brassicicolin A ($C_{20}H_{31}O_9$);[62] and gougeroxymycin.[63]

Several new synthetic antifungal agents have been reported. One of the most promising is diphenyl(4-chlorophenyl)-1-imidazolylmethane, 10, (BAY b 5097), an orally

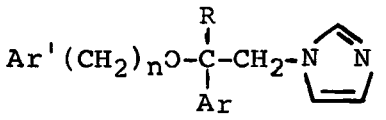


10

active substance that has in vitro activity against a range of organisms encompassing dermatophytes, chromomycetes, Aspergillus, Nocardia, Madurella, Candida, and dimorphic fungi.[64,65,66,67,68] Administered orally, the drug protected mice against candidosis, histoplasmosis, and aspergillosis. Absorption by various routes, as well as blood levels and urinary excretion, was explored in several animal species. Absorption of the drug was rapid after oral administration but poor after subcutaneous or intraperitoneal administration. Serum levels and concentrations of metabolites in blood and urine were determined in man. At levels attainable in vivo, the drug was fungistatic. Topical application was effective in experimental T. mentagrophytes and T. quinckeanum infections in mice and guinea pigs. Excellent results were obtained in humans with aspergillosis and candidal septicemia,

endocarditis, pneumonia, and pyelonephritis.

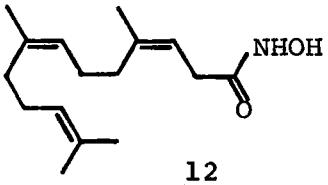
The preparation and antimycotic properties of a large number of derivatives of 1-phenethylimidazole were reported.[69] Several compounds with structure 11 had excellent *in vitro* activities against dermatophytes, *C. albicans*, and gram-positive bacteria. The most promising candidates are undergoing clinical evaluation.



(especially R= H, n= 1 and Ar and Ar' halogenated)

11

Homofarnesoyl hydroxamic acid, 12, has been reported[70] to be very potent topically against dermatophytes. Several tetrahydro-1,3,5-thiadiazine-2-thiones, 13, were synthesized and found to have antimycotic, antibacterial, and anthelmintic properties.[71] Strong *in vitro* antimycotic activity was found[72,73] for some 2-phenylpyridazone derivatives, 14.



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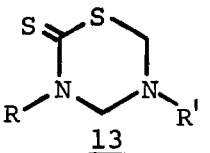

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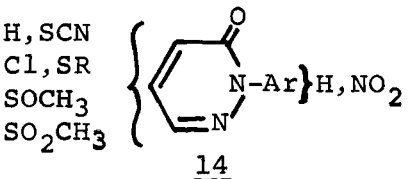
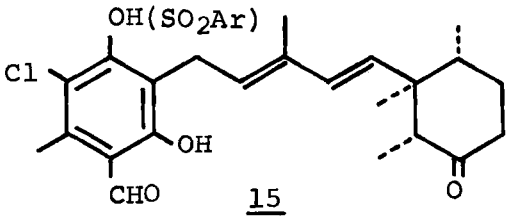


14

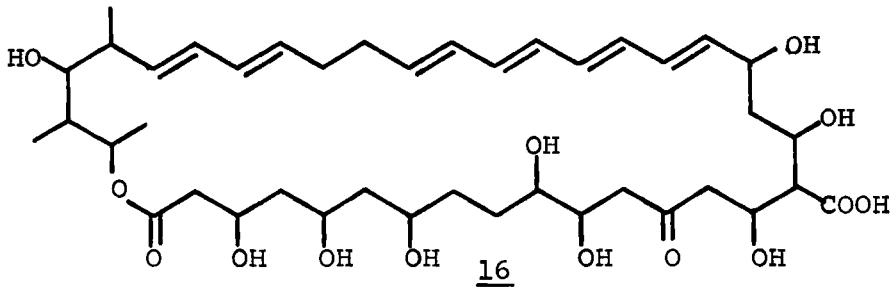
Activities against several fungi were measured for 3-amino-thiazoline-2-thiones and 4H-1,3,4-thiadiazines[74] and for some N-substituted amides and amine salts of sorbic acid.[75] *In vitro* activity against a broad spectrum of microorganisms, including *C. albicans*, was found for 17β-amino-3,5-androstadiene and 17β-amino-5-androstene.[76] Significant *in vitro* activity against *A. niger* and *C. albicans* was found for β-phenethyldithiocarbamates.[77] N-(α-Naphthyl)iodoacetamide, in a 1-2% ointment, gave a cure rate of 50% in experimental dermatophytosis and candidosis in guinea pigs.[78] Pyran copolymer (divinyl ether-maleic anhydride polyanion), an interferon inducer, protected mice against cryptococcosis. This substance has no *in vitro* antifungal activity, but induces a healthy carrier state; viable *C. neoformans* is found in the lung and brain at necropsy.[79]

Structural Work and Chemical Studies - The structure of ascochlorin, 15, has been determined by x-ray studies on the p-bromobenzenesulfonate.[80]



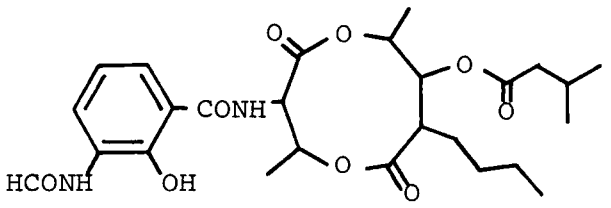
15

The detailed account of the structural determination of stendomycin has been published.[81] The structure and an abstract of the work were given in the 1968 Annual Reports. A partial structure for saramycetin has been proposed.[82] Mild acid hydrolysis produces a fragment with about half the molecular weight of the parent molecule and the amino acid sequence: glycyl-threonyl-saramycetic acid I-yl-cysteinyl-saramycetic acid II-yl-aspartyl-proline. A modified structure, 16, for the aglycone of nystatin has been proposed.[83] Mass spectral studies[84] on oligomycins, rutamycin, and their



16

acetates have shown that their molecular weight is about 800. The heptaenic macrolide, perimycin, gave N-methyl-p-aminoacetophenone after treatment with base, and the sugar, methyl perosaminide, after acid hydrolysis.[85] The levorin A complex of heptaenic aromatic macrolides has been resolved into four components by countercurrent distribution,[86,87] and the activities of the components against C. albicans and filamentous fungi and the toxicities for mice were determined.[88] The hexaene antibiotic, hexamycin, was characterized spectroscopically. It has a molecular weight of ca. 800. The in vitro activity against a number of fungi was determined.[89] Total syntheses of the dilactone antibiotics, antimycin A, 17, and avenaciolide, 18, have been reported.[90,91] Recent studies indicate that avenaciolide inhibits lipolysis in adipose cells



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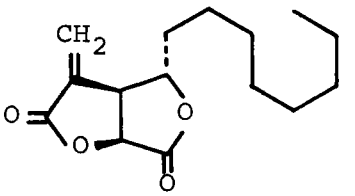
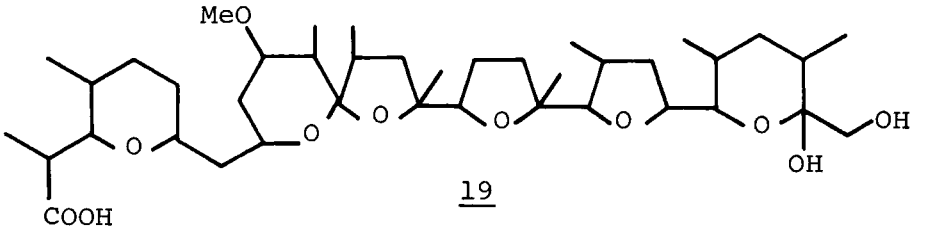


18

and abolishes the stimulation of glucose oxidation caused by insulin and proteolytic enzymes.[92] Identical structures, 19, have been determined for nigericin[93] and polyetherin A[94] by x-ray crystallography, and the identity of these with X-464 has been established by spectroscopic methods.[95]



19

Mode-of-Action Studies - An excellent review of the mode of action of the polyene antifungal antibiotics has been presented.[96] The interactions of polyene macrolides with membranes has been the subject of several studies. Amphotericin B increases permeability for small molecules in membranes containing cholesterol and phospholipid.[97] In erythrocytes, the increased efflux of potassium is offset by an increase in the membrane cation pump rate, which provides an explanation for the failure of amphotericin B to shorten the lifespan of erythrocytes *in vitro*.[98] The effect of sterols in membrane-polyene antibiotic interactions has been explored[99] in *Pythium* sp. PRL 2142. This organism lacks the ability to synthesize membrane sterol, but can incorporate exogenous sterol into protoplasmic membrane. Amphotericin B, nystatin, candidin, and candicidin have no effect on mycelia grown in the absence of sterol, but cause increased leakage, particularly of carbohydrate and protein components, from mycelia grown in the presence of sterol. Filipin and pimaricin mimic the effect of insulin in lowering levels of cyclic 3',5'-adenosine monophosphate (cyclic AMP) in fat cells by facilitating the leakage of cyclic AMP from them.[100] This effect is counteracted

by cholesterol. Amphotericin B reduced the ability of plasma membranes in the toad bladder to maintain intracellular ionic gradients, ultimately resulting in structural and functional disintegration. Detailed studies on morphologic changes in the cells were made.[101] Several experimental variables in the toad bladder-amphotericin B interaction were explored.[102] In a review of the mechanism of action of aldosterone, stimulation by amphotericin B of Na^+ transport in toad bladder was discussed briefly in relation to similar stimulation by aldosterone.[103]

Amphotericin B-treated endospores of Coccidioides immitis, a biphasic pathogenic fungus, showed no capacity for endogenous oxidation.[104] Cysteine reversed the effect of amphotericin B and restored normal endogenous respiration. However, the endospores were not capable of producing the tissue form, spherules, but allowed mycelial growth, the non-tissue form. It was suggested that this effect may be due to a reduced capacity of the cells to catabolize glucose.

Interesting findings have been reported on the effect of orally administered polyenes on sterol and steroid metabolism in animals. Candicidin, amphotericin B, and filipin, administered to dogs for 21 days at 5 to 20 mg/kg, reduced the size of the prostate and lowered serum cholesterol levels. Nystatin was less effective. Since very little polyene is absorbed from the gut, it is believed that the polyenes complex in the gut with dietary sterol and with sterol in the enterohepatic circulation. These complexes are then eliminated in the feces, thus pulling sterol and other lipids out of the body. The effect of polyene macrolides on prostatic size may be a consequence of the lowered level of androgen precursor, cholesterol.[105,106] In feeding studies in chicks, candicidin, perimycin, aureofungin, hamycin, filipin and amphotericin B caused a reduction in plasma cholesterol and, usually, an increase in fecal sterol.[107]

In another study, amphotericin B, given orally to dogs at 200 mg/day, resulted in decreased prostatic function. The effect could be prevented or reversed by administration of testosterone. Injection of human chorionic gonadotropin also caused partial reversal, possibly indicating involvement of the hypothalamic-pituitary complex. Circulating testosterone was reduced to an extremely low level in the dosed dogs. There was also a delayed degeneration of the seminiferous epithelial

component of the testes, resulting in cessation of spermatogenesis. The changes were reversible after withdrawal of the drug.[108]

The mode of action of pyrrolnitrin was investigated, using protoplasts of Bacillus megaterium, KM strain.[109] Pyrrolnitrin caused bursting of the protoplasts, but this effect could be neutralized stoichiometrically with certain phospholipids, e.g. phosphatidylethanolamine, indicating that pyrrolnitrin causes damage by combining with phospholipids in the cell membrane. Contrasting results were obtained in another study using Saccharomyces cerevisiae (whole cells, cell-free extracts, and mitochondria) and beef heart mitochondria.[110] It was concluded that the primary site in these systems was the terminal electron transport system between succinate or reduced nicotinamide adenine dinucleotide and coenzyme Q. No damage to cell membranes was observed.

REFERENCES

1. M. W. Falcone and V. F. Garagusi, S. Med. J., 62, 315 (1969).
2. F. C. Blodi, F. T. Hannah and J. A. C. Wadsworth, Amer. J. Ophthalmol., 76, 698 (1969).
3. M. W. Burn, Med. J. Aust., 1, 679 (1969).
4. D. B. Evans, Proc. Roy. Soc. Med., 62, 597 (1969).
5. D. G. Simpson, D. L. Brummer, A. D. Chaves, D. W. Cugell, H. A. Dickie, N. G. Hepper, A. K. Pierce and M. M. Ziskind, Amer. Rev. Resp. Dis., 100, 908 (1969).
6. D. D. Bindschadler and J. E. Bennett, J. Infec. Dis., 120, 437 (1969).
7. W. Perkins, Dis. Chest, 56, 389 (1969).
8. J. D. Parker, I. L. Doto and F. E. Tosh, Amer. Rev. Resp. Dis., 99, 895 (1969).
9. A. J. Atkinson, Jr. and D. D. Bindschadler, ibid., 99, 917 (1969).
10. W. R. Lockwood, F. Allison, Jr., B. E. Batson and J. F. Busey, ibid., 100, 314 (1969).
11. J. W. Polk, Dis. Chest, 56, 149 (1969).
12. F. F. Fountain and W. D. Sutliff, Amer. Rev. Resp. Dis., 99, 89 (1969).
13. P. J. Kozinn, C. C. Taschdjian, M. S. Seelig, L. Caroline, and A. Teitler, Sabouraudia, 7, 98 (1969).
14. G. L. Baum, R. L. Donnerberg and D. Stewart, New Eng. J. Med., 280, 410 (1969).
15. G. Kobayshi, L. N. Newmark, S. Wessler and L. V. Avioli,

J. Amer. Med. Ass., 210, 1741 (1969).
16. W. G. Cushard, Jr., M. Kohanim and L. I. Lantis, J. Bone Joint Surg., Amer. Vol. 51, 704 (1969).
17. C. E. Buckley, III, R. S. Mathews, J. A. Grant, W. G. Edwards and H. S. Neilsen, Arch. Intern. Med., 124, 748 (1969).
18. O. Olurin, A. O. Lucas and A. B. Oyediran, Amer. J. Ophthalmol., 68, 14 (1969).
19. C. Segal, C. G. Wheeler and R. Tompsett, New Eng. J. Med., 280, 206 (1969).
20. R. G. Slavin, D. J. Stanczyk and A. J. Lonigro, Amer. J. Med., 47, 306 (1969).
21. K. Watanabe and H. Ikemoto, Jap. J. Antibiot., 22, 155 (1969).
22. L. N. Mohapatra, K. Ramachandron, J. C. M. Shastry and B. N. Tandon, Indian J. Med. Res., 57, 128 (1969).
23. G. P. Bodey, Clin. Pharmacol. Ther., 10, 675 (1969).
24. S. Shadomy, H. J. Shadomy, J. A. McCay and J. P. Utz, Antimicrob. Ag. Chemother., 1968, 452 (1969).
25. S. Shadomy, H. J. Shadomy and J. P. Utz, Bacteriol. Proc., 111 (1969).
26. P. F. Cameron, Practitioner, 202, 695 (1969).
27. G. L. Mikhailets, S. L. Volinskaya, N. I. Ivanov and N. N. Slonitskaya, Antibiotiki, 14, 932 (1969).
28. N. D. Yarobkova, Ter. Arkh., 41, 106 (1969). Ringdoc Abstract 15839 J.
29. G. W. Lones, W. R. Piggott and W. B. Hill, Sabouraudia 6, 334 (1969).
30. L. J. Arthur, Proc. Roy. Soc. Med., 62, 906 (1969).
31. J. S. Vedder and W. F. Schorr, J. Amer. Med. Ass., 209, 1191 (1969).
32. R. A. J. Eady and S. Alexander, Practitioner, 203,670 (1969).
33. A. C. Ellison, E. Newmark and H. E. Kaufman, Amer. J. Ophthalmol., 68, 812 (1969).
34. H. Blank, D. Taplin and N. Zaias, Arch. Dermatol., 99, 135 (1969).
35. N. N. Slonitskaya, Antibiotiki, 14, 44 (1969).
36. L. Bessone, L. Anselmi and G. Villa Venzano, G. Ital. Dermatol., 110, 12 (1969).
37. R. S. Gordee and T. R. Matthews, Appl. Microbiol., 17, 690 (1969).
38. M. Ajisaki, K. Kariyone, K. Jomon, H. Yazawa and K. Arima, Agr. Biol. Chem., 33, 294 (1969).

39. J. P. Utz, In: "Systemic Mycoses", G. E. W. Wolstenholme and R. Porter, Ed., Little, Brown, Boston, p 247, (1968).
40. C. W. Emmons, ibid., p 267.
41. Anonymous, In: "Transactions of the 28th VA-Armed Forces Pulmonary Disease Research Conference", VA Department of Medicine and Surgery, Central Office, Washington, p 96, (1969).
42. T. Noto, M. Sawada, K. Ando and K. Koyama, J. Antibiot., 22, 165 (1969).
43. B. A. Davis, Obstet. Gynecol., 34, 40 (1969).
44. R. F. Pittillo and B. J. Ray, Appl. Microbiol., 17, 773 (1969).
45. J. S. Watkins, M. J. Campbell and D. Gardner-Medwin, Brit. Med. J., 3, 29 (1969).
46. M. J. Dultera, Jr., and S. Osterhout, S. Med. J., 62, 295 (1969).
47. G. A. Ellestad, R. H. Evans, Jr. and M. P. Kunstmann, J. Amer. Chem. Soc., 91, 2134 (1969).
48. S. Sugawara, 9th Intersci. Conf. Antimicrob. Agents Chemother. Abstrs., p 28 (1969).
49. M. Arai, K. Ishibashi and H. Okazaki, ibid., p 28 (1969).
50. A. E. Bird and A. C. Marshall, J. Chem. Soc. C, 2418 (1969).
51. M. Richards, A. E. Bird and J. E. Munden, J. Antibiot. 22, 388 (1969).
52. W. J. McGahren, J. H. Van den Hende and L. A. Mitscher, J. Amer. Chem. Soc., 91, 157 (1969).
53. G. M. Strunz, A. S. Court, J. Komlossy and M. A. Stillwell, Can. J. Chem., 47, 3700 (1969).
54. W. J. McGahren and M. P. Kunstmann, J. Amer. Chem. Soc., 91, 2808 (1969).
55. A. K. Dhar and S. K. Bose, Tetrahedron Lett., 4871 (1969).
56. Y. N. Shukla, J. S. Tandon, D. S. Bhakuni and M. M. Dhar, Experientia, 25, 357 (1969).
57. A. Kato, T. Sacki, S. Suzuki, K. Ando, G. Tamura and K. Arima, J. Antibiot., 22, 322 (1969).
58. G. M. Sharma, L. Michaels and P. R. Burkholder, ibid., 21, 659 (1968).
59. M. Ebata, K. Miyazaki and Y. Takahashi, ibid., 22, 467 (1969).
60. A. Ueda and Y. Sakagami, ibid., 22, 536 (1969).
61. S. Aizawa, Y. Nakamura, S. Shirato, R. Taguchi, I. Yamaguchi and T. Misato, ibid., 22, 457 (1969).
62. A. Ciegler and L. A. Lindenfelser, Experientia, 25, 719 (1969).

63. E. L. Wang, N. Kanda and H. Umezawa, J. Antibiot., 22, 211 (1969).
64. M. Plempel, K. Bartmann, K. H. Büchel and E. Regel, Deut. Med. Wochenschr., 94, 1356 (1969).
65. M. Plempel, K. Bartmann, K. H. Büchel and E. Regel, 9th Intersci. Conf. Antimicrob. Agents Chemother. Abstrs., p 33 (1969).
66. A. Freis, ibid., p 34 (1969).
67. W. N. Marget and D. Adam, ibid., p 34 (1969).
68. H. Oberste-Lehn, I. Baggesen and M. Plempel, Deut. Med. Wochenschr., 94, 1365 (1969).
69. E. F. Godefroi, J. Heeres, J. van Cutsem and P. A. J. Janssen, J. Med. Chem., 12, 784 (1969).
70. G. Coppi, C. C. Bonardi and R. Genova, Boll. Chim. Farm., 108, 421 (1969).
71. V. M. Schorr, W. Dürckheimer, P. Klatt, G. Lämmler, G. Nesemann and E. Schrinner, Arzneim.-Forsch., 19, 1807 (1969).
72. T. Takahashi, M. Takaya and Y. Maki, J. Pharm. Soc. Jap., 89, 1516 (1969).
73. T. Takahashi, M. Takaya and Y. Maki, ibid., 89, 1617 (1969).
74. Y. Usui and T. Yamano, ibid., 89, 699 (1969).
75. L. W. Morgan, D. H. Cronk and R. P. Knott, J. Pharm. Sci., 58, 942 (1969).
76. K. Yagishita and Y. Nagai, J. Pharm. Soc. Jap., 89, 1152 (1969).
77. W. O. Foye and J. C. Anderson, J. Pharm. Sci., 58, 1558 (1969).
78. H. Yamazoe, S. Takamura and Y. Tanaka, J. Pharm. Soc. Jap., 88, 1602 (1968).
79. A. Munson, S. Shadomy, A. McCay, W. Wooles and W. Regelson, 9th Intersci. Conf. Antimicrob. Agents Chemother Abstrs., p 54 (1969).
80. Y. Nawata, K. Ando, G. Tamura, K. Arima and Y. Iitaka, J. Antibiot., 22, 511 (1969).
81. M. Bodanszky, J. Izdebski and I. Muramatsu, J. Amer. Chem. Soc., 91, 2351 (1969).
82. A. Aszalos, J. Kirschbaum, O. Kocy, F. Russo-Alesi and J. Alicino, J. Antibiot., 22, 577 (1969).
83. D. G. Manwaring and R. W. Rickards, Tetrahedron Lett., 5319 (1969).
84. J. W. Chamberlin, M. Gorman and A. Agtarap, Biochem. Biophys. Res. Commun., 34, 448 (1969).
85. C. H. Lee and C. P. Schaffner, Tetrahedron, 25, 2229 (1969).

86. V. A. Tsyganov and E. P. Yakovleva, Antibiotiki, 14, 387 (1969).
87. V. A. Tsyganov and E. P. Yakovleva, ibid., 14, 505 (1969).
88. V. A. Tsyganov and E. P. Yakovleva, ibid., 14, 635 (1969).
89. K. Eisenbrandt, H. J. Müller and M. Klinkowski, Z. Allg. Mikrobiol., 8, 175 (1968).
90. M. Kinoshita, M. Wada and S. Umezawa, J. Antibiot., 22, 580 (1969).
91. W. L. Parker and F. Johnson, J. Amer. Chem. Soc., 91, 7208 (1969).
92. J. F. Kuo, I. K. Dill, C. E. Holmlund and N. Bohonos, Biochem. Pharmacol., 17, 345 (1968).
93. L. K. Steinrauf, M. Pinkerton and J. W. Chamberlin, Biochem. Biophys. Res. Commun., 33, 29 (1968).
94. T. Kubota, S. Matsutani, M. Shiro and H. Koyama, Chem. Commun., 1541 (1968).
95. A. Stempel, J. W. Westley and W. Benz, J. Antibiot., 22, 384 (1969).
96. J. O. Lampen, Amer. J. Clin. Pathol., 52, 138 (1969).
97. T. E. Andreoli, V. W. Dennis and A. M. Weigl, J. Gen. Physiol., 53, 133 (1969).
98. S. F. Blum, S. B. Shohet, D. G. Nathan and F. H. Gardner, J. Lab. Clin. Med., 73, 980 (1969).
99. J. J. Child, G. Défago and R. H. Haskins, Can. J. Microbiol., 15, 599 (1969).
100. J. F. Kuo and E. C. De Renzo, J. Biol. Chem., 244, 2252 (1969).
101. A. J. Saladino, P. J. Bentley and B. F. Trump, Amer. J. Pathol., 54, 421 (1969).
102. I. Singer, M. M. Civan, R. F. Baddour and A. Leaf, Amer. J. Physiol., 217, 938 (1969).
103. D. D. Fanestil, Annu. Rev. Med., 20, 223 (1969).
104. J. E. Sippel and H. B. Levine, Sabouraudia, 7, 159 (1969).
105. H. W. Gordon and C. P. Schaffner, Proc. Nat. Acad. Sci. U. S., 60, 1201 (1968).
106. C. P. Schaffner and H. W. Gordon, ibid., 61, 36 (1968).
107. H. Fisher, P. Griminger and C. P. Schaffner, Proc. Soc. Exp. Biol. Med., 132, 253 (1969).
108. J. H. Texter and D. S. Coffey, Invest. Urol., 7, 90 (1969).
109. M. Nose and K. Arima, J. Antibiot., 22, 135 (1969).
110. R. K. Tripathi and D. Gottlieb, J. Bacteriol., 100, 310 (1969).

Chapter 14. Antineoplastic Agents

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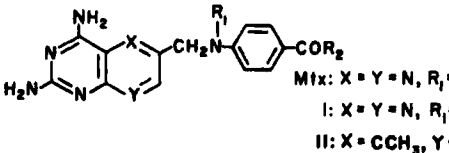
Treatments - Recent reviews on the management of human acute leukemias emphasize the importance of drug combinations, treatment schedules, and total cell eradication.[1-3] Failure of the digestive tract epithelium rather than of the bone marrow could be the limiting factor in intensive chemotherapy that appears so promising.[4] The fact that immunosuppression by antilymphocytic serum released noncycling tumor cells in mice[5] is of great interest, since solid tumors, which contain noncycling cells, are generally unresponsive to therapy, particularly with antimetabolites.

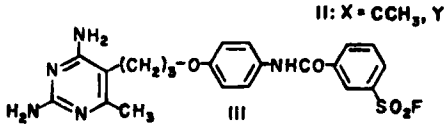
Agents. Folic Acid Antagonists - Studies on the transport energetics[6] of methotrexate (Mtx) and the effect of 5-formyltetrahydrofolate on its uptake by L1210 mouse leukemia cells[7] have been reported. The Mtx-resistant P1534 murine leukemia cells accumulated Mtx 13 times more slowly than the sensitive L1210 cells. In contrast, uptake of 4-amino-4-deoxy-N^{10}-methylpteroic acid (I), which appears to be taken up by a different process, proceeded at similar rates in both cell lines.[8] Studies in mouse liver supernatant[9] and in L-cells in culture[10] indicate that Mtx may have important sites of action—such as thymidylate synthetase[11]—in addition to its inhibition of folate and dihydrofolate reductase. But stimulation of thymidylate synthetase activity by Mtx in leukocytes of patients with acute granulocytic leukemia,[12] which may be due to stimulation of synthesis of the enzyme,[13] has been observed. Killing of L-cells in culture by Mtx results from the inhibition of thymidylic acid synthesis, while concurrent inhibition of purine synthesis tends to prevent cell killing. Also cells whose proliferation was inhibited prior to exposure to Mtx were resistant to the drug.[14] In keeping with these results is the observation that Mtx killed cells 6–7 times faster in the proliferating than in the resting state.[15] In a study of the effectiveness of various dosage schedules of Mtx in advanced breast carcinoma 38 of 96 patients responded to therapy.[16]



Mtx: X = Y = N, $R_1 = CH_3$, $R_2 = NHCH(CO_2H)(CH_2)_2CO_2H$

I: X = Y = N, $R_1 = CH_3$, $R_2 = OH$

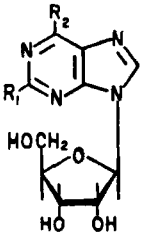
II: X = CCH_3, Y = CH, $R_1 = H$, $R_2 = -NHCH(CO_2H)CH_2CO_2H$



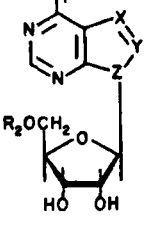
III

One (II) of a number of 2,4-diaminoquinazolines that inhibit dihydrofolic reductase[17] has a particularly high therapeutic index against L1210 leukemia in mice and is a candidate for clinical trials.[18] Because of difficulties encountered with the transport of active-site-directed irreversible inhibitors (such as III) of dihydrofolic reductase, the emphasis in this study has been shifted to the development of compounds that retain their specificity, but that are better able to passively diffuse into cells.[19,20]

Purines - The in vitro capacity for 6-mercaptopurine (MP) anabolism, by hypoxanthine phosphoribosyltransferase,[21] can be a useful predictive measure of drug responsiveness in man.[22] 6-(Methylthio)purine ribonucleoside (MeMPR) is also converted to its nucleotide (MeMPRP), which accumulates in human erythrocytes.[23] Conversion of MP to its nucleotide is enhanced by prior doses of MP[24] or by coadministration of MeMPR.[25] This enhancement of MP anabolism is thought to be the basis of the synergistic action of the combination of MP and MeMPR.[25] MeMPRP is the most potent known inhibitor of PRPP amidotransferase from adenocarcinoma 755 cells,[26] and complete reversal of the growth inhibitory effects of MeMPR at low but not high levels by hypoxanthine and 5-aminoimidazole-4-carboxamide (AIC) support the view that the amidotransferase is the most sensitive site of inhibition by this analog.[27]



MeMPR: $R_1 = H, R_2 = SCH_3$
IV: $R_1 = NH_2, R_2 = SH$
V: $R_1 = H, R_2 = NHCH_2CH{=}CH_2$
VI: $R_1 = H, R_2 = NHOH$

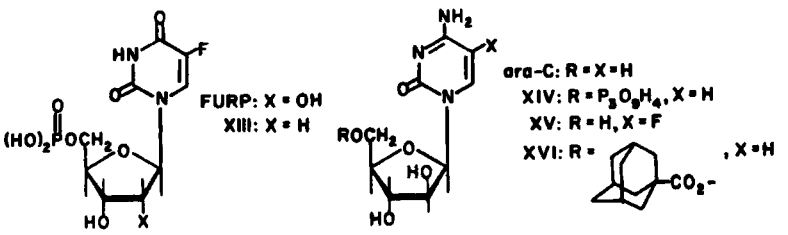


VII: $R_1 = OH, R_2 = H, X = Y = CH, Z = N$
VIII: $R_1 = NH_2, R_2 = -PO_3H_2, X = Y = CH, Z = N$
IX: $R_1 = NH_2, R_2 = H, X = Y = N, Z = CH$
X: $R_1 = OH, R_2 = H, X = Y = N, Z = CH$
XI: $R_1 = NH_2, R_2 = P_3O_9H_4, X = Y = N, Z = CH$
XII: $R_1 = OH, R_2 = H, X = Y = Z = N$

β-2'-Deoxythioguanosine (IV) is phosphorylated by extracts from solid tumors and from normal bone marrows, whereas α-2'-deoxythioguanosine is phosphorylated only by the extracts from certain neoplasms. The DNA polymerase of several tumors incorporated nucleotides of both the α- and β-anomers.[28] The synergistic effects against leukemia L1210 observed with the combination of 1-β-D-arabinofuranosylcytosine (ara-C) and thioguanine (TG) appear to be due in part to the subadditive toxicity of the combination,[29] which may result from a decrease in incorporation of TG into DNA caused by inhibition of the DNA polymerase of normal

mouse tissues by ara-C. However, TG ribonucleotide has been found to be a poor substrate for but a potent inhibitor of ATP-GMP phosphotransferase, which could explain the cytolytic action of TG.[30] A high remission rate in adult acute leukemia with the ara-C–TG combination has been observed.[31] N-Allyladenosine (V) appears to inhibit bacterial and mammalian cells by entirely different mechanisms.[32] Four other N-substituted adenosines are moderately active against leukemia L1210.[33] Hemolysis at low doses of N-hydroxyadenosine (VI) precluded a satisfactory clinical antileukemic trial of this agent.[34] Investigations of biologic activity of 7-deazainosine (VII) indicate that this analog must be converted to tubercidin anabolites (VIII) to exert its effects.[35,36] In tumor bearing mice formycin (IX) is extensively deaminated to the inosine analog (X), but there is also a significant conversion to formycin 5'-triphosphate (XI),[37] which may be the active form of this compound. The metabolism and cytotoxicity of 8-azainosine (XII), a nucleoside active against leukemia L1210 and adenocarcinoma 755 in mice, has been studied in a number of cell lines deficient in purine anabolizing enzymes.[38] A review of the metabolism and mechanisms of action of purine analogs[39] and a book dealing with the biochemistry of purine and pyrimidine analogs[40] have appeared.

Pyrimidines - 5-Fluorouracil (FU) can be converted to 5-fluorouridylic acid (FURP) by sequential enzymic addition of ribose and phosphate or via a pyrimidine 5'-phosphoribosyltransferase.[41] It appears that a determinant of cell responsiveness to FU is the production of 2'-deoxy-5-

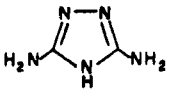


fluorouridylic acid (XIII) and that this nucleotide is formed primarily from FURP produced by the pyrimidine 5'-phosphoribosyltransferase pathway,[42] perhaps because of intracellular compartmentation.[43] Transfer RNA isolated from E. coli B grown in the presence of FU contains a mixture of normal and FU-containing transfer RNA's having 70–84% of the uracil residues replaced[44,45] and reduced levels of minor pyrimidine nucleosides.[46,47] The relationship, if any, between the incorporation of 5-fluorouracil into tRNA and rRNA[45,48] and its cytotoxicity and anticancer activity is not known. A ten-year study of the use of FU in disseminated breast cancer shows that increased survival time can be correlated with responsiveness to the drug.[49] FU significantly augments

radiation therapy in patients with locally inoperable gastrointestinal adenocarcinoma[50] and advanced cancer of the head and neck.[51]

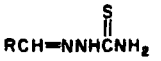
Acute leukemia blast cells vary widely in their ability to phosphorylate ara-C, which might explain the variability in the response of acute leukemias to the drug.[52] Acquired resistance to ara-C in L1210 leukemia, the frequency of which can be reduced by quinacrine hydrochloride,[53] has been attributed to a change in the affinity of the mutant DNA polymerase for ara-C triphosphate (XIV).[54] Ara-C[55,56] and 1-β-D-arabinofuranosyl-5-fluorocytosine (XV)[56] kill only cells in the S-phase of the mitotic cycle when exposed, both in vitro and in vivo. Cytotoxic effects of ara-C in the mouse are primarily on the proliferating zone of the intestinal crypts, and inhibition of DNA synthesis must be sustained for several hours in order to induce irreversible damage in sensitive S-phase cells.[56,57] A number of studies on the route of administration,[58,59] dose, and schedule[60-62] of ara-C in humans have been carried out in an attempt to optimize the anticancer effects of this drug. The effects of a single dose of 1-(5-O-adamantoyl-β-D-arabinofuranosyl)-cytosine (XVI), a "depot" form of ara-C, against leukemia L1210 are similar to those obtained with ara-C administered every 3 hr. for 24 hr.[63]

Other Antimetabolites - Guanazole (XVII) inhibits DNA synthesis in L1210 cells in vivo, probably due to its inhibition of ribonucleoside diphosphate reductase,[64] the enzyme that, under delicate allosteric control and in conjunction with thioredoxin, reduces uridine, cytidine, adenosine, and guanosine diphosphates to the corresponding 2'-deoxyribonucleoside diphosphates.[65] The activity against leukemia L1210 of this compound, hydroxyurea (HU) and a number of thiosemicarbazones (XVIII), all of which inhibit nucleotide reductase, shows the same schedule dependency that is exhibited by ara-C,[66] which inhibits DNA polymerase as well as the reductase, because of the S-phase specificity of these compounds.[67] Inhibition by HU of ribonucleotide reductase may result from the chelation of ferrous ions, although this could not be demonstrated directly.[68] Metabolism of HU[69] may be necessary before the chelation of ferrous ions is possible. Five of fourteen patients with metastatic renal cell carcinoma, which is usually resistant to chemotherapeutic agents, showed objective responses to HU lasting two to five months.[70]



XVII

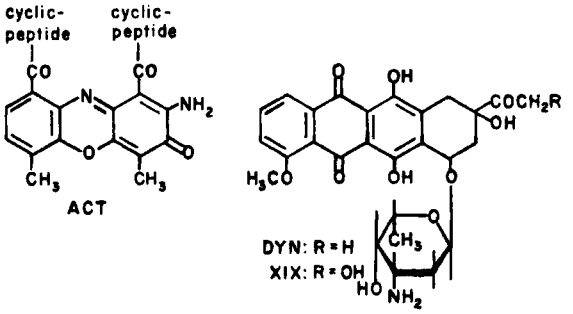
HU



XVIII

Crystalline L-asparaginase (Aase) from E. coli B has been prepared and evaluated in experimental animals.[71] The clearance or disappearance rate of injected Aase in mice infected with lactate dehydrogenase-elevating (LDH) virus is reduced by a factor of 5 to 10 as compared with normal mice, and all tested mouse leukemias responsive to Aase therapy were found to carry the LDH virus.[72] Antibodies produced by pretreatment of animals with Aase reduced the effectiveness of this enzyme against leukemia L5178Y in mice.[73] Synergism has been observed in animals with Aase and glutamine antagonists[74,75] possibly due to the L-glutaminase activity of Aase,[76] since glutamine is required for asparagine synthetase. The production of L-asparagine synthetase correlates with resistance to Aase in murine and human tumors.[77] Arginase has also been found to be cytotoxic to mouse tumor cells in culture.[78] Several clinical trials[79-82] show that Aase is most effective in acute lymphatic leukemia and acute myelogenous leukemia.[81] Aase toxicity, particularly CNS toxicity, may be a more serious problem than initially thought.[83,84]

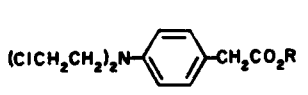
Inhibitors of RNA Synthesis - A detailed study of the binding of actinomycin D (ACT) to DNA indicated that the specificity for guanine among the common bases results from electronic interactions in the π-complex formed in an intercalated structure,[85] but the presence of guanine in DNA is not a sufficient requisite for the binding of ACT.[86] The uptake of tritiated daunomycin (DYN) by cells in culture was rapid and dependent only on drug concentration in the medium, and a quantitative relationship between uptake and the inhibition of uridine incorporation exists.[87] DYN appears to interfere with the cell cycle during the G2 period, thus delaying the onset of mitosis in cells that have already synthesized DNA.[88] DYN is an effective agent in acute childhood leukemia, but the optimal



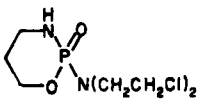
treatment schedule is not yet known.[89] Adriamycin (XIX) appears to have a better therapeutic index than DYN in experimental animal systems.[90] Although mithramycin (MYN) inhibited protein and DNA syntheses in cells in culture, these inhibitions were not dose related suggesting that the primary site of action was on RNA synthesis,[91] presumably because of complex formation of the antibiotic with DNA. MYN is effective against

testicular cancer,[92,93] but its administration has been accompanied by marked hemorrhagic diathesis.[94] The biologic activity of chromomycin A_3 and a series of analogs parallels their capacity to bind to DNA.[95]

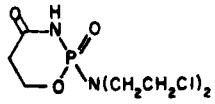
Chemically Reactive Compounds - If the alkylation of DNA by nitrogen mustard (HN2) is carried out at 0°, cross-linking is greatly reduced and labile intermediates accumulate. These results raise questions concerning an earlier proposal involving transalkylation from triester phosphates to purine ring nitrogens.[96] Selected single doses of HN2 or cyclophosphamide (CYM) caused a transient inhibition of growth of a plasmacytoma in hamsters, but it is not known whether the resumption of growth is a result of DNA repair or a result of killing only cells most sensitive to the agent.[97] Possible mechanisms of resistance to alkylating agents include decreased membrane permeability to the drug,[98,99] increased concentration of protective agents such as thiols,[100,101] and increased DNA repair. Compounds known to inhibit the repair mechanism increase the sensitivity of an alkylating-agent-resistant plasmacytoma to HN2 and CYM.[102] The most interesting of a number of new nitrogen mustard derivatives[103-106] is a series of steroid esters of p-[N, N-bis(2-chloroethyl)amino]phenylacetic acid (XX-XXIV), some of which are excellent inhibitors of a hormone-dependent DMBA-induced mammary adenocarcinoma.[106] The CYM activating enzyme has been identified as the mixed function oxidase system of liver microsomes responsible for the metabolism of drugs, steroids, and carcinogens,[107] and a proximate, biologically active metabolite of CYM has, at last, been isolated and identified as 4-oxo-CYM.[108] 4-Oxo-CYM may be of practical importance because of the relatively slow rate that humans metabolize CYM to it.[109] Generally favorable reports on the clinical use of CYM



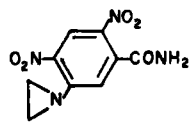
XX: R = Cholesterol
XXI: R = Estrone
XXII: R = Estradiol
XXIII: R = Testosterone
XXIV: R = Deoxycorticosterone



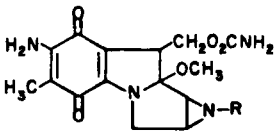
CYM



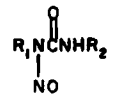
4-oxo-CYM



XXV



XXVI: R = H
XXVII: R = CH_3

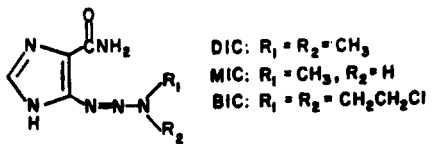


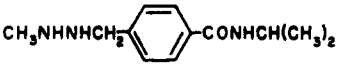
XXVIII: $R_1 = CH_3$, $R_2 = H$
BCNU: $R_1 = R_2 = CH_2CH_2Cl$
CCNU: $R_1 = CH_2CH_2Cl$, R_2 =

XXIX: $R_1 = CH_2CH_2Cl$, R_2 =

alone[110-114] and in combination with radiation,[115-117] and of HN2[118] and melphalan[119] have appeared. An aziridine, 2,4-dinitro-5-(1-aziridinyl)-benzamide (XXV), is one of the most active compounds ever tested against the Walker rat carcinoma 256.[120] A number of favorable clinical reports on mitomycin C (XXVI)[121-123] and porfiromycin (XXVII)[124,125] have appeared.

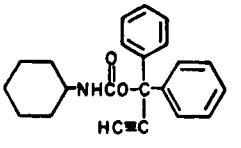
The mechanism of action of the nitrosoureas is not known, but they differ from the "classical" biological alkylating agents in a number of ways. 1-Methyl-1-nitrosourea (XXVIII) reacts with DNA by methylation but also by carbamoylation,[126] although these reactions have not as yet been correlated with the anticancer effect of this compound. Contrary to popular opinion, N-methyl-N-nitroso compounds do not methylate DNA via diazomethane.[127] Comparative studies of the effects of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and HN2 on the progression of cells through the cell cycle show that the biologic effects of these compounds differ in a number of respects.[128,129] Of a series of nitrosoureas highly effective against leukemia L1210 [including BCNU and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU)] only 1-(2-chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea (XXIX) cured mice with established Lewis lung tumors, a generally unresponsive animal neoplasm.[130] Studies on the toxicity of CCNU indicate that it induces qualitatively the same effects as BCNU and other nitrosoureas.[131,132] Clinical results with BCNU indicate a definite role for this drug in the treatment of Hodgkin's disease, lymphosarcoma, and reticulum cell sarcoma,[133] and it is the most effective agent now available for the treatment of human brain tumors.[134]



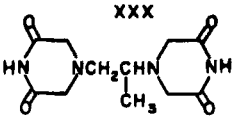


MIH

XXXI



XXX



XXXII

Patients given 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DIC) excrete large amounts of AIC[135,136] and DIC is N-demethylated by rat liver microsome to formate and AIC.[135] These facts coupled with the previous observations that 5-(3-methyl-1-triazeno)imidazole-4-carboxamide (MIC) decomposes to AIC in aqueous media and shows the same order of activity against leukemia L1210 as DIC,[137] indicate that N-demethylation of DIC to MIC could be essential to its activity. DIC is an effective agent for the treatment of metastatic melanoma and certain

other human tumors.[138,139] The synthesis and biological activity of the 5-triazenoimidazoles have been reviewed,[140] and the papers presented at the New York Academy of Sciences' symposium on the "Biological Effect of the Alkylating Agents" have been published.[141]

Miscellaneous Agents - Studies with sarcoma 180 cells indicate that incorporation of acetate into phospholipids is the most sensitive of a number of sites of action of vinleurosine.[142] This inhibition could be the basis[142] of the cytotoxicity or the neurotoxicity[143,144] of the vinca alkaloids. Vincristine is briefly effective in Hodgkin's disease and reticulum cell sarcoma (RCS), but not in carcinomas and nonlymphomatous sarcomas.[144-146] Procarbazine (MIH), the active metabolite of which appears to be an azo derivative,[147] is also effective against Hodgkin's disease[146] and RCS[146,148] and somewhat effective against bronchogenic carcinoma.[149] Some clinical activity has been observed with 1,1-diphenyl-2-propynyl cyclohexanecarbamate (XXX),[150,151] 1-acetyl-2-picolinoylhydrazine (XXXI),[152] and 1,2-bis(3,5-dioxopiperazin-1-yl)propane (XXXII).[153] The activity against experimental animal neoplasms of many types of structures—the bleomycins,[154] chloropromazine,[155] a new podophyllotoxin glucoside,[156] D-glucosamine,[157] DL-2-mercapto-3-hydroxypropanol,[158] camptothecin sodium,[159] mitomalcin,[160] and a number of plant products[161] has been reported. The mechanisms of action of selected tumor-inhibitory drugs and some new developments in cancer chemotherapy have been reviewed.[162]

Immunosuppressive Agents - The immunosuppressive activity of a variety of structural types including purine nucleosides,[163-165] DIC,[166] BIC,[167] compounds that complex with DNA,[168,169] benzimidazoleureas,[170] cinanserin,[171] mycophenolic acid,[172] alanosine,[173] L-asparaginase,[174] concanavalin A,[175] ovalicin,[176] chloroamphenicol analogs,[177] and methylcellulose[178] has been reported. AdOCA (XVI), presumably because of its "depot" action, is much superior to ara-C as an immunosuppressive agent.[179] Immunosuppression and carcinogenesis have been related.[180,181] An excellent review of the role of antimetabolites in immunosuppression and transplantation has appeared.[182]

References

1. E. S. Henderson, Seminars in Hematology, 6, 271 (1969).
2. C. L. Whitfield, J. R. Durant, and T. W. Sheehy, Clin. Med., 76, 14 (1969).
3. Symposium: A Critical Evaluation of Cancer Chemotherapy, Cancer Res., 29, 2255 (1969).
4. F. S. Philips, H. S. Schwartz, and S. S. Sternberg, in T. C. Hall and K. Karrer, Eds., "Pharmacological Principles in Antitumor Chemotherapy," Verlag der Wierner Medizinischen Akademie, Vienna, 1968, p. 84.
5. J. J. DeCosse and S. Gelfant, Science, 162, 698 (1968).
6. I. D. Goldman, J. Biol. Chem., 244, 3779 (1969).
7. A. Nahas, P. F. Nixon, and J. R. Bertino, Proc. Amer. Assoc. Cancer Res., 10, 64 (1969).
8. D. Kessel, Mol. Pharmacol., 5, 21 (1969).
9. P. T. Condit and T. Yoshino, Proc. Amer. Assoc. Cancer Res., 10, 15 (1969).
10. J. Borsa and G. F. Whitmore, Mol. Pharmacol., 5, 303 (1969).
11. J. Borsa and G. F. Whitmore, ibid., 5, 318 (1969).
12. D. Roberts, T. C. Hall, and D. Rosenthal, Cancer Res., 29, 571 (1969).
13. R. Labow, G. F. Maley, and F. Maley, ibid., 29, 366 (1969).
14. J. Borsa and G. F. Whitmore, ibid., 29, 737 (1969).
15. W. M. Hryniuk, G. A. Fischer, and J. R. Bertino, Mol. Pharmacol., 5, 557 (1969).
16. M. E. Sears, W. Tucker, C. A. Coltman, and J. Bonnett, Abstract 40, 5th Annual Scientific Meeting of the American Society of Clinical Oncology, Inc., San Francisco, March 1969.
17. D. J. Hutchison, F. M. Sirotnak, and A. M. Albrecht, Proc. Amer. Assoc. Cancer Res., 10, 41 (1969).
18. M. Shimoyama and D. J. Hutchison, ibid., 10, 80 (1969).
19. B. R. Baker, E. E. Janson, and N. M. J. Vermeulen, J. Med. Chem., 12, 898 (1969).
20. B. R. Baker and N. M. J. Vermeulen, ibid., 13, 82 (1970).
21. T. A. Krenitsky, R. Papaioannou, and G. B. Elion, J. Biol. Chem., 244, 1263 (1969).
22. D. Kessel and T. C. Hall, Cancer Res., 29, 2116 (1969).
23. T. L. Loo, D. H. W. Ho, D. R. Blossom, B. J. Shepard, and E. Frei, III, Biochem. Pharmacol., 18, 1711 (1969).
24. M. L. Meloni and W. I. Rogers, ibid., 18, 413 (1969).
25. A. R. P. Paterson and A. Moriwaki, Cancer Res., 29, 681 (1969).
26. D. L. Hill and L. L. Bennett, Jr., Biochemistry, 8, 122 (1969).
27. L. L. Bennett, Jr. and D. J. Adamson, Biochem. Pharmacol., 19, 000 (1970).
28. A. Peery and G. A. LePage, Cancer Res., 29, 617 (1969).
29. L. H. Schmidt, J. A. Montgomery, W. R. Laster, Jr., and F. M. Schabel, Jr., Proc. Amer. Assoc. Cancer Res., 11, 70 (1970).
30. R. P. Miech, R. York, and R. E. Parks, Jr., Mol. Pharmacol., 5, 30 (1969).
31. T. S. Gee, K.-P. Yu, and B. D. Clarkson, Cancer, 23, 1019 (1969).
32. S. Straight and D. L. Hill, Proc. Amer. Assoc. Cancer Res., 11, 76 (1970).
33. M. H. Fleysher, A. Bloch, M. T. Hakala, and C. A. Nichol, J. Med. Chem., 12, 1056 (1969).
34. I. H. Krakoff and M. R. Dollinger, Proç. Amer. Assoc. Cancer Res., 10, 47 (1969).
35. A. Bloch, E. Mihich, R. J. Leonard, and C. A. Nichol, Cancer Res., 29, 110 (1969).
36. E. Mihich, C. L. Simpson, and A. I. Mulhern, ibid., 29, 116 (1969).
37. I. C. Caldwell, J. F. Henderson, and A. R. P. Paterson, Can. J. Biochem., 47, 901 (1969).
38. P. W. Allan and L. L. Bennett, Jr., Proc. Amer. Assoc. Cancer Res., 11, 2 (1970).
39. R. W. Brockman, "Metabolism and Mechanisms of Action of Purine Analogues," A Collection of Papers Presented at the Twenty-Second Annual Symposium on Fundamental Cancer Research, 1968, The Williams and Wilkins Company, Baltimore, 1968.
40. M. E. Balis, "Antagonists and Nucleic Acids," Wiley Interscience, John Wiley & Sons, Inc., New York, 1968.
41. P. Reyes, Biochemistry, 8, 2057 (1969).
42. D. Kessel, T. C. Hall, and P. Reyes, Mol. Pharmacol., 5, 481 (1969).
43. P. Reyes and T. C. Hall, Biochem. Pharmacol., 18, 2587 (1969).
44. I. I. Kaiser, Biochemistry, 8, 231 (1969).
45. J. L. Johnson, K. R. Yamamoto, P. O. Weislogel, and J. Horowitz, ibid., 8, 1901 (1969).
46. I. I. Kaiser, Biochim. Biophys. Acta, 182, 449 (1969).
47. I. I Kaiser, M. Jacobson, and C. Hedgcoth, J. Biol. Chem., 244, 6707 (1969).
48. U. Stenram and R. Willen, Experientia, 24, 909 (1968).
49. F. J. Ansfield, G. Ramirez, S. Mackman, G. T. Bryan, and A. R. Curreri, Cancer Res., 29, 1062 (1969).
50. C. G. Moertel, D. S. Childs, R. J. Reitemeier, M. Y. Colby, M. A. Holbrook, and

R. G. Hahn, Proc. Amer. Assoc. Cancer Res., 10, 60 (1969).
51. F. J. Ansfield, H. Vermund, and F. F. Gallin, Abstract 1, 5th Annual Scientific Meeting of the American Society of Clinical Oncology, Inc., San Francisco, March 1969.
52. D. Kessel, T. C. Hall, and D. Rosenthal, Cancer Res., 29, 459 (1969).
53. M. K. Bach, ibid., 29, 1881 (1969).
54. M. K. Bach, ibid., 29, 1036 (1969).
55. M. Karon and S. Shirakawa, ibid., 29, 687 (1969).
56. L. Lenaz, S. S. Sternberg, and F. S. Philips, ibid., 29, 1790 (1969).
57. W. B. Leach, W. R. Laster, Jr., J. G. Mayo, D. P. Griswold, Jr., and F. M. Schabel, Jr., ibid., 29, 529 (1969).
58. J. Z. Finklestein and M. Karon, Proc. Amer. Assoc. Cancer Res., 10, 25 (1969).
59. H. Savel and S. L. Burns, Cancer Chemother. Rept. (Part 1), 53, 153 (1969).
60. G. P. Bodey, E. J. Freireich, R. W. Monto, and J. S. Hewlett, ibid., 53, 59 (1969).
61. M. E. Nesbit, Jr., Proc. Amer. Assoc. Cancer Res., 10, 64 (1969).
62. E. Frei III, J. N. Bickers, J. S. Hewlett, M. Lane, W. V. Leary, and R. W. Talley, Cancer Res., 29, 1325 (1969).
63. G. L. Neil, R. C. Manak, and P. F. Wiley, Proc. Amer. Assoc. Cancer Res., 10, 64 (1969).
64. R. W. Brockman, S. Shaddix, W. R. Laster, Jr., and F. M. Schabel, Jr., to be submitted.
65. N. C. Brown and P. Reichard, J. Mol. Biol., 36, 25, 39 (1969).
66. H. E. Skipper, F. M. Schabel, Jr., and W. S. Wilcox, Cancer Chemother. Rept., 51, 125 (1967).
67. H. E. Skipper, personal communication.
68. E. C. Moore, Cancer Res., 29, 291 (1969).
69. H. S. Rosenkranz, R. D. Pollak, and R. M. Schmidt, ibid., 29, 209 (1969).
70. H. B. Nevinny and T. C. Hall, J. Clin. Pharmacol., 8, 352 (1968).
71. P. K. Ho and G. A. Poore, Proc. Amer. Assoc. Cancer Res., 10, 39 (1969).
72. V. Riley, Nature, 220, 1245 (1968).
73. S. Vadlamudi, M. Padarathsingh, V. S. Waravdekar, and A. Goldin, Proc. Amer. Assoc. Cancer Res., 10, 95 (1969).
74. S. P. Jacobs, I. Wodinsky, C. J. Kensler, and J. Venditti, ibid., 10, 43 (1969).
75. G. S. Tarnowski, I. M. Mountain, and C. C. Stock, ibid., 10, 92 (1969).
76. H. K. Miller, J. S. Salser, and M. E. Balis, Cancer Res., 29, 183 (1969).
77. C. M. Haskell and G. P. Canellos, Proc. Amer. Assoc. Cancer Res., 10, 36 (1969).
78. A. F. Burton, ibid., 10, 12 (1969).
79. R. L. Capizzi, R. Peterson, D. A. Cooney, W. A. Creasey, and R. E. Handschumacher, ibid., 10, 12 (1969).
80. J. E. Harris, J. P. Whitecar, G. P. Bodey, and E. J. Freireich, ibid., 10, 35 (1969).
81. L. Tallal, C. Tan, H. Oettgen, M. McCarthy, L. Helson, and L. Murphy, ibid., 10, 92 (1969).
82. J. M. Hill, E. Loeb, A. MacLellan, A. Khan, J. Roberts, W. F. Shields, and N. O. Hill, Cancer Res., 29, 1574 (1969).
83. G. P. Canellos, C. M. Haskell, J. Arseneau, and P. P. Carbone, Cancer Chemother. Rept. (Part 1), 53, 67 (1969).
84. C. M. Haskell, G. P. Canellos, B. G. Leventhal, P. P. Carbone, A. A. Serpick, and H. H. Hansen, Cancer Res., 29, 974 (1969).
85. W. Muller and D. M. Crothers, J. Mol. Biol., 35, 251 (1968).
86. R. D. Wells, Science, 165, 75 (1969).
87. A. Rusconi and A. DiMarco, Cancer Res., 29, 1507 (1969).
88. J. Whang-Peng, B. G. Leventhal, J. W. Adamson, and S. Perry, Cancer, 23, 113 (1969).
89. N. A. Shore, Proc. Amer. Assoc. Cancer Res., 10, 80 (1969).
90. A. Di Marco, M. Gaetani, and B. Scarpinato, Cancer Chemother. Rept. (Part 1), 53, 33 (1969).
91. G. Northrop, S. G. Taylor, III, and R. L. Northrop, Cancer Res., 29, 1916 (1969).
92. R. A. Oberfield, R. D. Sullivan, and W. Dube, Abstract 32, 5th Annual Scientific Meeting of the American Society of Clinical Oncology, Inc., San Francisco, March 1969.
93. B. J. Kennedy, Abstract 20, 5th Annual Scientific Meeting of the American Society of Clinical Oncology, Inc., San Francisco, March 1969.
94. R. W. Monto, R. W. Talley, M. J. Caldwell, W. C. Levin, and M. M. Guest, Cancer Res., 29, 697 (1969).
95. T. Hayasaka and Y. Inoue, Biochemistry, 8, 2342 (1969).
96. R. J. Rutman, E. H. L. Chun, and J. Jones, Biochim. Biophys. Acta, 174, 663 (1969).

97. G. P. Wheeler and J. A. Alexander, Cancer Res., 29, 98 (1969).
98. E. H. L. Chun, L. Gonzales, F. S. Lewis, J. Jones, and R. J. Rutman, ibid., 29, 1184 (1969).
99. O. Klatt, J. S. Stehlin, Jr., C. McBride, and A. C. Griffin, ibid., 29, 286 (1969).
100. M. K. Wolpert and R. W. Ruddon, ibid., 29, 873 (1969).
101. G. J. Goldenberg and C. L. Vanstone, Proc. Amer. Assoc. Cancer Res., 10, 30 (1969).
102. D. Gaudin and K. L. Yielding, Proc. Soc. Exp. Biol. Med., 131, 1413 (1969).
103. G. Csaba and J. Korosi, Neoplasma, 15, 4 (1968).
104. S. Vickers, P. Hebborn, J. F. Moran, and D. J. Triggle, J. Med. Chem., 12, 491 (1969).
105. Y. F. Shealy and R. F. Struck, ibid., 12, 907 (1969).
106. M. E. Wall, G. S. Abernethy, Jr., F. I. Carroll, and D. J. Taylor, ibid., 12, 810 (1969).
107. J. L. Cohen and J. Y. Jao, Proc. Amer. Assoc. Cancer Res., 10, 14 (1969).
108. D. L. Hill, M. C. Kirk, and R. F. Struck, J. Amer. Chem. Soc., 92, 000 (1970).
109. L. B. Mellett, S. M. El Dareer, J. K. Luce, and E. Frei, III, The Pharmacologist, 11, 273 (1969).
110. I. J. Ertel, L. Samuels, and W. A. Newton, Jr., Proc. Amer. Assoc. Cancer Res., 10, 22 (1969).
111. M. H. Cohen, J. M. Bennett, C. W. Berard, J. L. Ziegler, C. L. Vogel, J. N. Sheagren, and P. P. Carbone, Cancer, 23, 1259 (1969).
112. J. Z. Finklestein, R. E. Hittle, and G. D. Hammond, ibid., 23, 1239 (1969).
113. Z. Saba, T. C. Hall, and C. T. Griffiths, ibid., 23, 1122 (1969).
114. C. A. Perez, T. J. Vietti, L. V. Ackerman, and W. E. Powers, Gen. Practice, 40, 78 (1969).
115. N. Wollner and J. H. Burchenal, Proc. Amer. Assoc. Cancer Res., 10, 100 (1969).
116. W. L. Wilson, J. G. de la Garza, R. B. Green, and L. C. Montemayer, Abstract 51, 5th Annual Scientific Meeting of the American Society of Clinical Oncology, Inc., San Francisco, March 1969.
117. R. Johnson and S. R. Humphreys, Cancer, 23, 161 (1969).
118. J. Solomon, E. M. Jacobs, Jr., and J. R. Bateman, Proc. Amer. Assoc. Cancer Res., 10, 85 (1969).
119. J. Brook, J. R. Bateman, and J. L. Steinfeld, ibid., 10, 9 (1969).
120. L. M. Cobb, T. A. Connors, L. A. Elson, A. H. Khan, B. C. V. Mitchley, W. C. J. Ross, and M. E. Whisson, Biochem. Pharmacol., 18, 1519 (1969).
121. T. Neyazaki, M. Ikeda, Y. Seki, N. Egawa, and C. Suzuki, Cancer, 24, 912 (1969).
122. F. J. Ansfield, Cancer Chemother. Rept. (Part 1), 53, 287 (1969).
123. A. E. Evans, R. M. Heyn, M. E. Nesbit, and J. R. Hartmann, ibid., 53, 297 (1969).
124. T. B. Grage, A. J. Weiss, D. B. Rochlin, and V. Reynolds, Abstract 13, 5th Annual Scientific Meeting of the American Society of Clinical Oncology, Inc., San Francisco, March 1969.
125. R. C. Boronow and R. G. Borromeo, Cancer Chemother. Rept. (Part 1), 53, 305 (1969).
126. A. M. Serebnayni, M. A. Cmotoayeva, K. A. Kruglaykova, and R. G. Kostaynovskiy, Dokl. Akad. Sci. SSSR, 185, 817 (1969).
127. W. Lijinsky, J. Loo, and A. E. Ross, Nature, 218, 1174 (1968).
128. G. P. Wheeler, B. J. Bowdon, D. J. Adamson, and M. H. Vail, Cancer Res., 30, 000 (1970).
129. G. P. Wheeler, B. J. Bowdon, D. J. Adamson, and M. H. Vail, ibid., 30, 000 (1970).
130. F. M. Schabel, Jr., personal communication.
131. U. H. Schaeppi, M. M. Mason, and Z. Hadidian, Proc. Amer. Assoc. Cancer Res., 10, 76 (1969).
132. O. S. Selawry and H. H. Hansen, ibid., 10, 78 (1969).
133. V. B. Rege and R. E. Lenhard, Jr., Abstract 36, 5th Annual Scientific Meeting of the American Society of Clinical Oncology, Inc., San Francisco, March 1969.
134. C. B. Wilson, E. B. Boldrey, and K. J. Enot, Cancer Chemother. Rept., 54, 000 (1970).
135. J. L. Skibba, G. Ramirez, D. D. Beal, R. O. Johnson, and G. T. Bryan, Proc. Amer. Assoc. Cancer Res., 10, 83 (1969).
136. G. E. Housholder and T. L. Loo, Life Sci., 8, (Part 1), 533 (1969).
137. Y. F. Shealy and C. A. Krauth, J. Med. Chem., 9, 35 (1966).
138. J. K. Luce and W. G. Thurman, Proc. Amer. Assoc. Cancer Res., 10, 53 (1969).
139. J. L. Skibba, G. Ramirez, D. D. Beal, and G. T. Bryan, Cancer Res., 29, 1944 (1969).
140. Y. F. Shealy, J. Pharm. Sci., 59, 000 (1970).
141. New York Acad. Sci., 163, 589 (1969).
142. W. A. Creasey, Biochem. Pharmacol., 18, 227 (1969).

143. L. M. Slater, R. A. Wainer, and A. A. Serpick, Cancer, 23, 122 (1969).
144. B. C. Korbitz, H. L. Davis, Jr., G. Ramirez, and F. J. Ansfield, Cancer Chemother. Rept. (Part 1), 53, 249 (1969).
145. D. Desai, E. Z. Ezdinli, and L. Stutzman, Proc. Amer. Assoc. Cancer Res., 10, 18 (1969).
146. F. A. Flatow, J. E. Ultmann, G. A. Hyman, and F. M. Muggia, Cancer Chemother. Rept. (Part 1), 53, 39 (1969).
147. A. T. Huang and W. B. Kremer, Proc. Amer. Assoc. Cancer Res., 10, 41 (1969).
148. D. C. Stolinsky, J. Solomon, R. P. Pugh, A. R. Stevens, E. M. Jacobs, L. E. Irwin, D. A. Wood, J. L. Steinfeld, and J. R. Bateman, ibid., 10, 88 (1969).
149. M. L. Samuels, W. V. Leary, and C. D. Howe, Cancer Chemother. Rept. (Part 1), 53, 135 (1969).
150. C. B. Pratt and J. J. Wang, Proc. Amer. Assoc. Cancer Res., 10, 70 (1969).
151. J. Louis and F. Bull, ibid., 10, 52 (1969).
152. K. B. Olson, J. Horton, K. L. Pratt, W. J. Paladine, Jr., T. Cunningham, J. Sullivan, H. Hosley, and D. H. Treble, Cancer Chemother. Rept. (Part 1), 53, 291 (1969).
153. K. Hellmann, K. A. Newton, D. N. Whitmore, I. W. F. Hanham, and J. V. Bond, Brit. Med. J., 1, 822 (1969).
154. M. Takeuchi and T. Yamamoto, J. Antibiot., 21, 631 (1968).
155. M. H. Van Woert and S. H. Palmer, Cancer Res., 29, 1952 (1969).
156. H. Stahelin, Proc. Amer. Assoc. Cancer Res., 10, 86 (1969).
157. J. G. Bekesi, Z. Molnar, and R. J. Winzler, Cancer Res., 29, 353 (1969).
158. M. A. Apple and D. M. Greenberg, Cancer Chemother. Rept. (Part 1), 53, 195 (1969).
159. A. M. Guarino, L. G. Hart, J. B. Call, and V. T. Oliverio, Proc. Amer. Assoc. Cancer Res., 10, 33 (1969).
160. T. J. McBride, M. Axelrod, W. P. Cullen, W. S. Marsh, K. V. Rao, and C. S. Sodano, ibid., 10, 56 (1969).
161. S. M. Kupchan, Trans. N. Y. Acad. Sci., 32, 85 (1970).
162. A. C. Sartorelli and W. A. Creasey, Ann. Rev. Pharmacol., 9, 51 (1969).
163. R. H. Gisler and J. P. Bell, Biochem. Pharmacol., 18, 2115 (1969).
164. R. H. Gisler and J. P. Bell, ibid., 18, 2123 (1969).
165. B. Hacker and T. Feldbush, Proc. Amer. Assoc. Cancer Res., 10, 34 (1969).
166. C. Vogel, ibid., 10, 96 (1969).
167. C. L. Vogel, V. T. DeVita, R. P. Lisak, and M. W. Kies, Cancer Res., 29, 2249 (1969).
168. L. D. Zeleznick, J. A. Crim, and G. D. Gray, Biochem. Pharmacol., 18, 1823 (1969).
169. D. E. Griswold and E. M. Uyeki, Eur. J. Pharmacol., 6, 56 (1969).
170. C. J. Paget, K. Kisner, R. L. Stone, and D. C. DeLong, J. Med. Chem., 12, 1010 (1969).
171. J. Krapcho, R. C. Millonig, C. F. Turk, and B. J. Amrein, J. Med. Chem., 12, 164 (1969).
172. A. Mitsui and S. Suzuki, J. Antibiot., 22, 358 (1969).
173. D. Fumarola, Boll. Ist. Sieroter. Milan., 48, 184 (1969).
174. R. S. Schwartz, Nature, 224, 275 (1969).
175. H. Markowitz, D. A. Person, G. L. Gitnick, and R. E. Ritts, Jr., Science, 163, 476 (1969).
176. S. Lazary and H. Staehelin, Antibiot. Chemother., 15, 177 (1969).
177. A. S. Weisberger and T. M. Daniel, Proc. Soc. Exp. Biol. Med., 131, 570 (1969).
178. S. Benko, F. Biliczki, F. Durszt, and M. Csanady, Kiserl. Orvostud., 21, 6 (1969).
179. G. D. Gray, M. M. Mickelson, and J. A. Crim, Biochem. Pharmacol., 18, 2163 (1969).
180. O. Stutman, Science, 166, 620 (1969).
181. G. Parmiani, M. I. Colnaghi, and G. Della Porta, Proc. Soc. Exp. Biol. Med., 130, 828 (1969).
182. G. H. Hitchings and G. B. Elion, Accounts Chem. Res., 2, 202 (1969).

Chapter 15. Mechanism of Action of Antibiotics

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Introduction - A chapter on the mechanisms of action of antibiotics has been included for the first time in these Annual Reports. Thus, references to a number of relevant works on the subject will be included although some of them have appeared in the literature before 1969.

Antibacterial antibiotics can be broadly classified according to their specific site and mode of action as inhibitors of (1) respiration and (or) oxidative phosphorylation, (2) cell-wall mucopeptide synthesis, (3) cell membrane function, (4) nucleic acid synthesis, (5) protein synthesis, and (6) miscellaneous antibiotics.

Antibiotics of group (1) are equally toxic to all living organisms and consequently cannot be used clinically. Since the cell-wall mucopeptide is only present in bacteria and blue-green algae, antibiotics of group (2) are widely used clinically because of their low toxicity to higher organisms. Most antibiotics of group (3) are equally toxic to bacteria and higher organisms. Thus only a few (the polymyxins and tyrocidines) have some limited use clinically in infections by Gram negative bacteria. Despite their toxicity to man the polyene antibiotics (also included in group (3)) are used medically as antifungal agents. Most antibiotics of group (4) by interacting with DNA, block the growth of viruses, bacteria, higher organisms and tumor cells and are used as antitumor agents (mitomycins, porfiromycins, carzinophilin, phleomycin, streptonigrin, actinomycins, olivomycin and the antracycline antibiotics). The rifamycins, streptovaricin and streptolydigin (also included in group (4)) interact directly with bacterial RNA polymerase but not with the mammalian enzyme thus being very useful clinically. Rifampicin (a derivative of a rifamycin) is specially important as an antitubercular, anti-viral and anti-trachomal drug. Protein synthesis inhibitors acting on bacterial ribosomes (ribosomes of the 70 S type) (tetracyclines, chloramphenicol, streptomycin, neomycin, kanamycin, gentamycin, viomycin, lincomycin, the macrolides and the streptogramins) are used clinically as antibacterial agents. Protein synthesis inhibitors acting on ribosomes of higher cells (ribosomes of the 80 S type) (cycloheximide, tenuazonic acid, sparsomycin, gougerotin, puromycin and amicetin) have been tentatively used as antitumor agents. Among the antibiotics of group (6) novobiocin is clinically used as an antibacterial agent and griseofulvin as an antifungal compound. Antibiotics blocking biosynthesis of nucleic acid precursors because of their potential toxicity are mainly used as antitumor agents and occasionally as antiviral agents.

Reviews - Relevant reviews have appeared in the last few years on the subject dealing specifically with the mechanism of action of different antimicrobial drugs and antibiotics[1, 2], inhibitors of the bacterial ribosome[3], inhibitors of protein and nucleic acid synthesis[4] and inhibitors as tools in cell research[5].

Inhibitors of respiration and (or) oxidative phosphorylation - The oligomycins, rutamycin and aurovertin are known to inhibit phosphorylation at the step of the transfer of inorganic phosphate from a phosphorylated intermediate to ADP[6a, b]. However aurovertin, unlike the oligomycins does not inhibit ATPase induced by an uncoupling agent[7a,b] and, in fact, inhibits oligomycin stimulated phosphorylation[8a]. Studies on the effect of oligomycin on EDTA particles from mitochondria are consistent with those obtained previously in intact mitochondria[8b].

Antimycin A and usnic acid inhibit respiration at a site in the terminal electron transport system between cytochromes b and c_1[9,10]. The binding of antimycin A to cytochrome c_1, at a molar ratio 1/1, causes complete inhibition of Complex III of the respiratory chain[11]. However, the inhibition by antimycin A can be reversed by gramicidin. Also the activity of antimycin A treated Complex III can be restored by extraction of the antibiotic bound to that complex with diethyl ether[13].

The antibiotic gramicidin A, an uncoupler of phosphorylation in bacteria and in mammalian mitochondria[14], reverses the effect of oligomycin[12]. The antibiotic piericidin A is known to inhibit the NADH to cytochrome segment of the respiratory chain[15]. The structure of piericidin A resembles that of coenzyme Q and so it has been suggested that the antibiotic acts at a site in which coenzyme Q is involved. Piericidin A loses considerable activity if the side chain is replaced by a carboxyl group or if the ring hydroxyl is acetylated[16].

Inhibitors of cell wall mucopeptide synthesis - The antibiotic O-carbamyl-D-serine is a competitive inhibitor of alanine racemase, whereas D-cycloserine inhibits both D-alanyl-D-alanine synthetase and alanine racemase, both enzymes being required for synthesis of the peptide moiety of the mucopeptide[17].

Recent work utilizing ultraviolet absorption techniques has shown that vancomycin and ristocetin bind in cell-free systems to mucopeptide precursors in equimolar proportion and that the D-alanyl-D-alanine moiety of the peptide is required for the binding reaction[18]. Penicillin, considered an analogue of acyl-D-alanyl-D-alanine[19a,b], also modifies the absorption spectrum of vancomycin[18]. Studies on binding of ^{125}I-vancomycin in intact bacteria have confirmed the results on binding to cell-walls

in cell-free systems and have also shown that initially the antibiotic binds mainly to the cell-wall fraction but that lengthening the incubation time causes an increase in membrane binding. The specific peptide moiety of the mucopeptide competes with cell walls for vancomycin[20]. Vancomycin and ristocetin inhibit the D-alanine carboxypeptidase[21,22] by binding to the terminal -D-alanyl-D-alanine moiety of the mucopeptide. The antibiotics might also inhibit the transpeptidase reaction, although this has not been demonstrated due to experimental difficulties[20].

Penicillins and cephalosporins are known to inhibit the two final steps in mucopeptide synthesis catalysed by transpeptidase and carboxypeptidase[19a,b]. Using purified preparations of the enzymes, it was shown that penicillin is an extremely effective competitor of the natural substrate of carboxypeptidase[21]. It binds very firmly to the particular enzyme, which contains transpeptidase, inactivating it[22].

Bacitracin, a cyclic peptide, has been shown to block mucopeptide synthesis by inhibiting the dephosphorylation of a lipid pyrophosphate intermediate to lipid phosphate. It prevents the lipid carrier from further participating in the reaction cycle of peptidoglycan synthesis causing accumulation of the lipid pyrophosphate[23a,b]. The antibiotics enduramycin[24a] (a basic polypeptide), moenomycin[24b] and prasinomycin[24c] (two phosphorus containing antibiotics) are also inhibitors of mucopeptide synthesis. However, the specific reaction inhibited by these three antibiotics is still not well known.

The chemical structure of penicillin-amido-β-lactam hydrolases from different bacteria has been studied by amino-acid sequence determinations. A genetic relationship among different bacterial genera was unveiled[25a].

The compound 3,6-bis(dimethylaminomethyl) catechol is a powerful catalyst of penicillin hydrolysis. Many of the characteristics of its catalytic activity resemble those observed with a number of hydrolytic enzymes (pH optimum, presence of an intermediate, structural specificity necessary for optimum activity, basicity of the amine, susceptibility of the β-lactam to nucleophilic attack and role of the penicillin side chain in the interaction with the catalyst). The results obtained explain satisfactorily the high resistance level of cephalosporins to penicillinase[25b].

Studies on the chemical aspects of penicillin allergy have shown that the penicilloyl determinant in penicillin allergy can be formed by the reaction of benzylpenicillenic acid with free functional groups in proteins. Benzylpenicillenic acid can react with SH groups of protein to form thioesters similar to its reaction with amino groups to form amides[26a]. Products obtained in aminolysis and enzymic hydrolysis of cephalosporins

have been analyzed by chemical methods and proton-magnetic-resonance-techniques. Some of the identified products are postulated as determinant in cephalosporin allergy[26b,c]. An excellent review on the chemical aspects of penicillin allergy has appeared recently[26d].

Inhibitors affecting the cell membrane - In this group a number of basic polypeptide antibiotics including polymyxin B, colistin A, circulin A, the tyrocidines and gramicidin S are included. These are surface active agents which disorganize the cell membrane altering a number of its functions[27a,b].

Several research groups have concentrated their efforts in the study of the mode of action of the antibiotics known generically as ionophores (so named because of their ability to carry ions across natural and artificial lipid barrier systems through formation of complexes). The mode of action of these antibiotics has been reviewed recently[28a-d]. The three subclasses comprising the ionophorous antibiotics are the valinomycin group, the nigericin group and the alamethicin group. The valinomycin group includes the antibiotics valinomycin, enniantins A and B, the macrotetrolide actins (nonactin, monactin, dinactin and trinactin) and gramicidins A, B and C; these antibiotics do not have ionizable groups and tend to establish an electrochemical equilibrium across the membrane[28a-d]. The nigericin group, nigericin and monensin, have a single ionizable carboxyl and establish an exchange diffusion equilibrium across a lipid barrier between the various cationic species, which can be complexed, and protons which can be transported by the carboxyl group[28a-d]. The third group includes alamethicin which has also a dissociable carboxyl group; in the case of alamethicin the complex formation center is maintained cyclic by covalent bonds and the complexes formed are not dependent on the pH[29a,b]. X-ray analysis of the crystalline nonactin-K^+ complex has shown that the K-ion is surrounded by eight oxygen atoms. The whole complex resembles a ball with a lipophilic exterior[30]. In the enniantin B-K^+ complex, the K-ion is surrounded by six carbonyl oxygen atoms in octahedral coordination and the complex can be described as a disc with a lipophilic exterior[31]. The complex valinomycin-K^+ appears to be built along the lines of enniantin B-K^+, K^+ having coordination with six carbonyl oxygen atoms[32]. The silver salts of the nigericin group have also a very similar structure[33]. The effect of the ionophorous antibiotics has also been investigated in bacterial chromatophores and it was found that antibiotics of the valinomycin group increase the rate and magnitude of the light-induced external pH rise, whereas antibiotics of the nigericin group decrease the light-induced external pH rise[34].

Other antibiotics, known to interfere with membrane function, are the polyene antibiotics which include nystatin, amphotericin A, filipin, candidin and others. However, these antibiotics are active only on membranes

containing sterols, such as the membranes of higher cells. Since bacterial cell membranes do not have sterols the polyene antibiotics are not active against bacteria[35].

Inhibitors of nucleic acid synthesis - The mode of action of antibiotics included in this group has been repeatedly reviewed recently[36a-d]. Some of these reviews deal mainly with antibiotics which inhibit preferentially DNA synthesis by binding to the DNA (mitomycins, porfiromycins, carzinophilin, phleomycin and streptonigrin) and those which inhibit preferentially RNA synthesis, by binding to the DNA. The latter group includes the antracycline antibiotics (ruticulomycin A, nogalamycin, cinerubin A and B, daunomycin and isoquinocycline), echinomycin, olivomycin, mithramycin, actinomycins and chromomycin A_3[36a-c]. One review is mainly concerned with the rifamycins which bind to the bacterial RNA-polymerase specifically, inhibiting the synthesis of RNA without affecting the synthesis of DNA[36d].

Studies on binding of actinomycin to DNA led to a new model proposing that the actinomycin chromophore is intercalated into the DNA adjacent to a GC base-pair[37]. Studies on the activity of the chromomycins have shown that the dissociation rates of their complexes with DNA increase in the order of the decreasing size of the sugar side chains[38]. Phleomycin has been shown to compete with Hg^{++} for binding to the carbonyl group in 2 position of thymidine[39a,b]. The structure of the antibiotic granaticin has recently been elucidated[40]. It was shown that this antibiotic and also streptonigrin preferentially inhibit RNA synthesis, although they also block the synthesis of DNA[36c]. The effect of echinomycin and olivomycin on RNA synthesis in Ehrlich ascites tumour cells has been studied recently. It was found that these antibiotics preferentially inhibit the synthesis of ribosomal RNA[41a]. Binding of chromomycin to DNA and its inhibitory effect on DNA and RNA have been studied. It was found that one molecule of the antibiotic is bound per four nucleotide base pairs[41b]. Kanchanomycin inhibits DNA and RNA polymerases by binding to DNA. Studies on binding of the antibiotic to DNA, RNA and polynucleotides have shown that Mg^{++} is required for the binding[41c,d].

A number of papers have appeared in the last few years on the mode of action of rifamycins, streptovaricin and streptolydigin. Several workers found that in cell-free systems the rifamycins selectively block bacterial RNA polymerase without affecting RNA polymerase from higher organisms[42,43,44a-c]. A similar selectivity occurs in the case of the antibiotics streptovaricin[45] and streptolydigin[46a,b]. These antibiotics, like the rifamycins, do not act on the template but directly on the RNA polymerase. This was demonstrated by the existence of bacterial mutants having RNA polymerase resistant to the rifamycins and streptovaricin[47-52].

Furthermore, in the case of rifampicin (=rifampin, a synthetic rifamycin derivative) the resistance was localized in the β-subunit of the RNA-polymerase[53]. Binding of rifamycin to RNA-polymerase from bacterial sensitive strains has been found under varied experimental conditions, while the antibiotic does not bind to the enzyme from resistant strains[54]. Rifamycin does not prevent the interaction of RNA-polymerase with template DNA[55]. The antibiotic blocks the addition of the first ribonucleotide at the initiation of synthesis of an RNA chain by RNA-polymerase, but it cannot inhibit the synthesis of an RNA chain once it has been started[56, 57]. On the other hand, streptolydigin appears to inhibit elongation of RNA synthesis[46a,b]. RNA-polymerase from rat liver mitochondria has also been shown to be sensitive to rifamycin[58]. The RNA-polymerase from blue-green algae was found to be sensitive to rifamycin, but to a lesser extent than the bacterial enzyme[59a,b].

It was found that the macrocyclic ring of rifamycin was responsible for the complex formation of the antibiotic with E. coli RNA polymerase and for the inhibitory effect of the antibiotic on the enzyme. Changes in other parts of the molecule have little effect on the direct action on E. coli RNA polymerase but may affect its permeability characteristics[60a]. Some derivatives of rifamycin, prepared by substitution with cyclic secondary amines, displayed activity against rifampicin resistant mutants of Staphylococcus aureus[60b].

Rifampicin is also an inhibitor of the synthesis of a number of phages and viruses. It has been demonstrated that the RNA polymerase, which transcribes phage β 22 following infection of Bacillus subtilis, retains the rifampicin sensitivity of the host cell enzyme[61a]. Rifampicin also inhibits the formation of infectious vaccinia virus and viral particles[61b]. Whereas virion formation is completely inhibited neither the synthesis of RNA and protein nor the activity of in vitro RNA polymerase associated to the virion is affected[61b]. Rifampicin inhibits the multiplication of poxvirus in vitro and in vivo. The side chain of this antibiotic derivative appears to be essential for the anti-viral effect and anti-trachomal activity found in monkeys[62a,b].

Inhibitors of protein synthesis - Antibiotics acting on the smaller ribosome subunit - Quite a number of antibiotics, including the tetracyclines, streptomycin, neomycin, kanamycin, paramomycin, gentamycin, hygromycin B, viomycin, edeine and spectinomycin, are known to block bacterial protein synthesis by acting on the 30 S subunit of the ribosome[3, 63].

A recently described animoglycosidic antibiotic, kasugamycin, is known to inhibit amino acid incorporation in a ribosomal system[64]. By obtaining 23 S ribonucleoprotein cores from kanamycin E. coli sensitive and

resistant strains it was possible to localize the site of action of this antibiotic on the 23 S core of the 30 S ribosome subunit[65].

Inhibition by tetracycline of F-methionyl-tRNA binding to ribosomes was considered a proof that initiation by F-met-tRNA takes place through binding to the "A" ribosomal site[66]. Studies on tetracycline binding to ribosomes have convinced the author that the interaction is possible with RNA, noncovalent and perhaps lacks physiological significance[67a]. Observations on the effect of varied tetracycline antibiotics on binding of aminoacyl-tRNA to ribosomes and on aminoacid incorporation have provided evidence that inhibition by all tetracycline antibiotics stems from their effect on aminoacyl-tRNA binding to ribosomes[67b].

For several years it was thought that aminoglycoside antibiotics block protein synthesis by causing a misreading of mRNA at the ribosome level. Investigations carried out in the last two years have clearly shown that the misreading effect of streptomycin and probably of other aminoglycoside antibiotics, on protein synthesis is artifactually obtained by using homopolynucleotides as mRNA in cell-free systems[68,71,72]. Misreading is not the main target "in vivo" or "in vitro" using natural mRNA. However, there is not complete agreement on the specific reaction inhibited by the antibiotic. One group of workers concluded that streptomycin modifies the association between 30 S and 50 S particles to yield aberrant initiation complexes[68]. To support their conclusion, they have presented data showing that inhibition of protein synthesis occurs to a greater extent with exogenous mRNA than with endogenous mRNA[69] and also that accumulation of monosomes occurs in bacteria treated with streptomycin[70]. Other workers have concluded that streptomycin acts by distorting the recognition site, thus causing impairment of the effective binding to the A site of both aminoacyl-tRNA and peptidyl-tRNA; polypeptide chain extension is inhibited and the peptidyl-tRNA blocked at the P-site[71,72]. Genetic evidence has also shown clearly that the streptomycin locus and the ribosomal ambiguity locus[73,74] are not the same.

Preparation of 16S RNA from streptomycin sensitive and resistant strains of E. coli and reconstitution of hybrid 30 S particles from the separated proteins made possible the localization of the effect of streptomycin in the protein fraction, either by studying amino acid incorporation[75] or by binding of the radioactive streptomycin[76,77]. Complete reconstitution of 30 S particles has been achieved by sequential addition to 16 S RNA of the separated proteins. Hence, it has been established that the protein controlled by the streptomycin locus is one of the separated proteins, known as the P 10 protein[74,78].

Likewise, reconstitution of 30 S subunits from 16 S RNA and 30 S

ribosomal proteins has made it possible to pin-point the 30 S ribosomal protein altered in spectinomycin resistance[79a,b].

Resistance to and dependence on streptomycin occur in a single gene and mutations to streptomycin dependence and its revertant are closely linked[80,81].

Co-resistance to neomycin and kanamycin takes place by mutations affecting the same gene (nek locus), which appears to specify a component of the 30 S ribosome subunit[82]. Spectinomycin gene and streptomycin gene are two other loci that also affect the 30 S ribosome subunit[83,84]. Ample experimental evidence has shown that the nek, spectinomycin and streptomycin genes specify three different ribosomal proteins[83,85]. When a mutation to neomycin resistance (nek locus) is introduced into cells resistant to spectinomycin, some of the double mutant strains are sensitive to spectinomycin, and the masking effect of neomycin mutations on the spectinomycin mutation was found to be exerted at the level of the ribosomes[86]. Transduction studies with a 50 S subunit assembly defective mutant showed that the locus affected is closely linked to the spectinomycin locus[87a]. Contrary to other 30 S ribosomal mutations kasugamycin resistance is located in E. coli in a cluster near the leucine region[87b], rather distant from that of streptomycin region.

Pactamycin interferes with the formation and stability of the initiating complex consisting of N-acetyl-phenylalanyl-tRNA, ribosome and poly U, possibly altering its structure[88a]. This effect is localized at the 30 S ribosome subunit and it was also observed in the course of a study of the binding of Ac-phe-tRNA to the 30 S ribosome subunit[88b].

Antibiotics acting on the larger ribosome subunit - The effects of specific inhibitors of peptide bond formation by either 70 S ribosomes (amicetin, sparsomycin, gougerotin and antibiotics of the chloramphenicol, macrolide, streptogramin A and lincomycin groups) or by 80 S ribosomes (amicetin, sparsomycin, gougerotin and anisomycin) have been reviewed recently[63].

Antibiotics known to act on the 50 S ribosome subunit include amicetin, sparsomycin, gougerotin and those of the chloramphenicol, macrolide, streptogramin A, streptogramin B and lincomycin groups[63].

Studies on the effect of a number of antibiotics on chloramphenicol binding to ribosomes, and on reconstitution of protein synthetic activity by hybrid ribosomes from sensitive and resistant subunits, were carried out to confirm that the site of action of amicetin, sparsomycin, blasticidin S and streptogramin B is on the 50 S ribosome subunit[89a]. Confirmation of the inhibitory effect of two antibiotics of the streptogramin family on

protein synthesis has been reported recently[89b]. The effects of a number of antibiotics on binding of Ac-phe-tRNA and formation of Ac-phe-puromycin has been confirmed[89c,d]. Antibiotics acting on 70 S ribosomes have been studied as to their effect on polylysyl-tRNA and Ac-phe-tRNA nonenzymic binding to ribosomes and on peptide bond formation using as a donor in the "P" site either polylysyl-tRNA or Ac-phe-tRNA. The results differed considerably depending on the substrate used for the experiments[90a]. Enzymic binding of phenylalanyl-tRNA to ribosomes was enhanced in the presence of gougerotin, blasticidin S and puromycin[90b].

Sparsomycin markedly stimulates and stabilizes the binding of N-acetyl-phenylalanyl-tRNA to ribosomes[91a]. In a more resolved system, it was shown that the antibiotic induces the formation of an inert complex between the 50 S ribosome subunit and the CCA-peptidyl moiety of the peptidyl donor substrate. This effect of sparsomycin is blocked by a number of inhibitors of peptidyl transferase including chloramphenicol, carbomycin, spiramycin III, streptogramin A and lincomycin[91b]. Gougerotin and amicetin have an effect rather similar to that of sparsomycin[63]. Direct studies on binding of aminoacyl-oligonucleotide have shown that chloramphenicol, sparsomycin, amicetin, gougerotin, celesticetin, carbomycin, streptogramin A, lincomycin and spiramycin III inhibit the interaction of that substrate with the "A" site of the 50 S ribosome subunit[92-94]. On the other hand, the antibiotics streptogramin A, spiramycin III, carbomycin and lincomycin inhibit the binding of CACCA-Leu-Ac to the "P" site of the 50 S ribosome subunit[63,95], whereas anisomycin blocks its binding to the "P" site of 60 S ribosome subunits[63].

Binding studies have confirmed the specificity of erythromycin interaction with the 50 S ribosome subunit, the requirement of monovalent ions and the lack of binding by 42 S nucleoprotein cores derived from the 50 S particles[96a,b]. Lincomycin inhibition of peptide bond formation has been confirmed whereas it was concluded that erythromycin inhibits translocation by acting on the 50 S subunit whereas streptomycin inhibits this step by acting on the 30 S subunit[96c].

Peptidyl transferase activity in 80 S ribosomes has been localized at the 60 S ribosome subunit and found to be specifically inhibited by anisomycin, amicetin, gougerotin and sparsomycin[97a,b].

Previous reports that, in eukaryotic cells chloramphenicol specifically inhibits protein synthesis by mitochondrial ribosomes, whereas cycloheximide blocks protein synthesis by cytoplasmic ribosomes were confirmed by a number of groups[98a-c].

The effect of several antibiotics acting at the ribosome level has been studied on bacterial polyribosome metabolism by a number of workers.

It was found that some antibiotics block polyribosome formation, whereas others block protein synthesis but allow polyribosome formation[99a-d].

Other inhibitors of protein synthesis - The initial finding that fusidic acid inhibits protein synthesis by specifically blocking the step of translocation at the level of the bacterial ribosome[100] was also confirmed using ribosomes of the 80 S type[101a,b]. It has been found recently that the antibiotic does not inhibit formation of the ribosome-G factor-GDP complex[101c]. On the other hand, cycloheximide and streptovitacin A were found to inhibit specifically protein synthesis only by 80 S ribosomes. The antibiotics block the translocation step by inactivating the enzyme involved in it[101d,e].

The antibiotics enomycin and phenomycin have been found to inhibit protein synthesis by specific interaction with ribosomes of the 80 S type but not of the 70 S type[102a,b]. On the other hand, bottromycin and berninamycin are only active with the 70 S ribosomes[100a,b], and actinobolin with both[103c].

Miscellaneous antibiotics - Griseofulvin, the sideromycins and novobiocin known to affect varied processes in bacteria , can be included in this group[2]. Also to be included are a number of antibiotics such as hadacidin, psicofuranine, tubercidin, toyocamycin, sangivamycin, azasering, diazo-oxo-norleucine (DON), cordycepin and mycophenolic acid known to block biosynthesis of some nucleic acid precursors[2].

It has been reported recently that mycophenolic acid inhibits DNA synthesis by fibroblasts. This is due to an effect of the antibiotic on an early stage of the biosynthesis of purine nucleotides blocking the enzyme IMP-NAD oxidoreductase[104]. Toyocamycin and tubercidin were found to cause accumulation in mammalian cells of the 45 S RNA precursor of the 28 S and 18 S ribosomal RNA[105]. The antibiotic sangivamycin was shown to be phosphorylated by enzyme extracts from mouse liver. Sangivamycin triphosphate was shown to be a substrate for ribonucleotide reductase but not for deamination[106].

References

1. Sixteenth Symposium of the Society for General Microbiology on "Biochemical Studies of Antimicrobial Drugs" Ed. B. A. Newton and P. E. Reynolds, Cambridge, University Press (1966).
2. Antibiotics. Vol. I, Mechanism of Action, Ed. D. Gottlieb and P. D. Shaw. Springer-Verlag, Berlin-New York (1967).
3. B.Weisblum and J.Davies, Bact.Rev., 32, 493 (1968).
4. Macromolecules: Biosynthesis and function. Ed. S.Ochoa, C.F.Heredia, C.Asensio and D.Nachmansohn, Academic Press (1970).
5. Inhibitors Tools in Cell Research, Ed. Th.Bücher and H.Sies, Springer-Verlag, Berlin-Heidelberg-New York (1969).

6. (a) P.D.Shaw, ref. 2, p. 585; (b) B.Bulos and E.Racker, J.Biol.Chem., 243, 3891 (1968).
7. (a) E.C.Slater and H.F.Ter Weller, ref. 5, p. 258; (b) H.Lardy and Chen-Ho Chieu Lin, ref. 5, p. 279.
8. (a) C.P.Lee and L.Ernster, European J.Biochem. 3, 391 (1968); (b) C.P. Lee, L.Ernster and B.Chance, European J.Biochem., 8, 153 (1969).
9. J. S. Rieske, ref. 2, p. 542.
10. P.D.Shaw, ref. 2, p. 611.
11. J.S.Rieske, H.Baum, C.D.Stoner and S.H.Lipton, J.Biol.Chem., 242, 4854 (1967).
12. J.L.Howland, Biochim.Biophys.Acta 153, 309 (1968).
13. Z.Kaniuga, J.Bryla and E.C.Slater, ref. 5, p. 282.
14. F.E.Hunter and L.S.Schwartz, ref. 2, p. 642.
15. P.A.Light, C.I.Ragan, R.A.Clegg and P.B.Garland, FEBS Letters, 1, 4 (1968).
16. M.Jeng, C.Hall, F.L.Crane, N.Takahashi, S.Tamura and K.Folkers, Biochemistry, 7, 1311 (1968).
17. F.C.Neuhaus, ref. 2, p. 40.
18. H.R.Perkins, Biochem.J., 111, 195 (1969).
19. (a) J.L.Strominger, ref. 5, p. 187; (b) J.L.Strominger, ref.2, p. 705.
20. H.R.Perkins and M.Nieto, Biochem.J., 116, 83 (1970).
21. K.Izaki and J.L.Strominger, J.Biol.Chem., 243, 3193 (1968).
22. K.Izaki, M.Matsuhashi and J.L.Strominger, J.Biol.Chem., 243, 3180(1968).
23. (a) G.Siewert and J.L.Strominger, Proc.Nat.Acad.Sci.U.S., 57, 767 (1967); (b) G.Siewert, ref. 5, p. 210.
24. (a) T.Tsuchiya and Y.Takeuchi, J.Antibiotics 21, 426 (1968); (b) G. Huber and G.Nesemann, Biochem.Biophys.Res.Commun. 30, 7 (1968); (c) E.Meyers, G.J.Miraglia, D.A.Smith, H.I.Basch, F.E.Pansy, W.H.Trejo and R.Donovick, Appl.Microbiol., 16 (4), 603 (1968).
25. (a) R.P.Ambler and R.J.Meadway, Nature, 222, 24 (1969); (b) R.D.Kenget and M.A.Schwartz, J.Pharm.Sci., 58, 1102 (1969).
26. (a) E.S.Wagner, W.W.Davis and M.Gorman, J.Med.Chem., 12, 483 (1969); (b)J.M.T.Hamilton-Miller, G.G.F.Newton and E.P.Abraham, Biochem.J., 116, 371 (1970); (c) J.M.T.Hamilton-Miller, E.Richards and E.P.Abraham, Biochem.J., 116, 385 (1970); (d) M.A.Schwartz, J.Pharm.Sci., 58, 643 (1969).
27. (a) O.K.Sebek, ref. 2, p. 142; (b) F.E.Hunter and L.S.Schwartz, ref. 2, p. 636.
28. (a) B.C.Pressman, In "The energy level and metabolic control in mitochondria", Ed. S.Papa, J.M.Tager, E.Quagliariello and E.C.Slater, p.87. Adriatica Editrice, Bari (1969); (b) B.C.Pressman and D.H.Haynes, In "The molecular basis of membrane function", Ed. D.C.Tosteron. Prentice Hall, New York (1969); (c) B.C.Pressman, In "Mitochondrial membranes", Ed. E.Racker, Reinhold, New York (1969); (d) W.Simon, L.A.R.Pioda and H.K.Wipf, ref. 5, p. 356.
29. (a) C.E.Meyer and F.Reusser, Experientia 23, 85 (1967); (b) B.C.Pressman, Fed. Proc. 27, 1283 (1968).
30. B.T.Kilbourn, J.D.Dunitz, L.A.R.Pioda and W.Simon, J.Mol.Biol., 30, 559 (1967).
31. M.Dobler, J.D.Dunitz and J.Krajewski, J.Mol.Biol., 42, 603 (1969).
32. M.Pinkerton, L.K.Steinrauf and P.Dawkins, Biochem.Biophys.Res.Commun., 35, 512 (1969).

33. L.K.Steinrauf, M.Pinkerton and J.W.Chamberlin, Biochem.Biophys.Res. Commun., 33, 29 (1968).
34. M.Nishimura and B.C.Pressman, Biochemistry, 8, 1360 (1969).
35. S.C.Kinsky, ref. 2, p. 122.
36. (a) G.Hartmann, W.Behr, K.A.Beissner, K.Honikel and A.Sippel, Angew. Chemie Int. Edition, 7, 693 (1968); (b) M.J.Waring, Nature, 219, 1320 (1968); (c) H.Kersten and W.Kersten, ref. 5, p. 11; (d) U.Lill, R.Santo, A.Sippel and G.Hartmann, ref. 5, p. 48.
37. W.Müller and D.M.Crothers, J.Mol.Biol., 35, 351 (1968).
38. W.Behr, K.Honikel and G.Hartmann, Europ.J.Biochem., 9, 82 (1969).
39. (a) P.Pietsch and H.Garret, Nature, 219, 488 (1968); (b) P.Pietsch and C.Corbert, Nature, 219, 933 (1968).
40. W.Keller-Schierlein, M.Buffrani and S.Bascza, Helv.Chim.Acta., 51, 1257 (1968).
41. (a) V.V.Mackedonski, FEBS Letters, 5, 73 (1969); (b) W.Behr, K.Honikel and G.Hartmann, European J.Biochem., 9, 82 (1969); (c) P.A.Friedman, P.B.Joel and I.H.Goldberg, Biochemistry, 8, 1535 (1969); (d) P.A.Friedman, T.K.Li and I.H.Goldberg, Biochemistry, 8, 1545 (1969).
42. G.Hartmann, K.O.Honikel, F.Knüsel and J.Nüesh, Biochim.Biophys.Acta, 145, 843 (1967).
43. W.Wehrli, J.Nüesh, F.Knüsel and M.Staehelin, Biochim.Biophys.Acta, 157, 215 (1968).
44. (a) H.Umezawa, M.Staoshi, H.Yamazaki and K.Nitta, J.Antibiotics, 21, 234 (1968); (b) J.M.Wilhelm, N.L.Oleinick and J.W.Corcoran, Biochim. Biophys.Acta, 166, 268 (1968); (c) E.Wintersberger and U.Wintersberger, FEBS Letters, 6, 58 (1970).
45. S.Mizuno. H.Yamazaki, K.Nitta and H.Umezawa, J.Antibiotics, 21, 66(1968).
46. (a) R.Schleif, Nature, 223, 1068 (1969); (b) C.Siddhikol, J.W.Erbstoeszer and B.Weisblum, J.Bact., 99, 151 (1969).
47. K.Nitta, S.Mizuno, H.Yamazaki and H.Umezawa, J.Antibiotics, 21,521(1968).
48. G.Tocchini-Valentini, P.Marino and A.Colvill, Nature, 220, 275 (1968).
49. D.Ezekiel and J.Hutchins, Nature, 220, 276 (1968).
50. W.Wehrli, F.Knüsel and M.Staehelin, Biochem.Biophys.Res.Commun., 32, 284 (1968).
51. C.Babinet and H.Condamine, Comp.Rend.Acad.Sci.(Paris), 267 D, 231 (1968).
52. T.Yura and K.Igarashi, Proc.Nat.Acad.Sci.U.S., 61, 1313 (1968).
53. D.Rabussay and W.Zillig, FEBS Letters, 5, 104 (1969).
54. W.Wehrli, F.Knüsel, K.Schmid and M.Staehelin, Proc.Nat.Acad.Sci,U.S., 61, 667 (1968).
55. V.Neuhoff, W.B.Schill and H.Sternbach, Hoppe-Zeylers Z.Physiol.Chem., 350, 335 (1969).
56. E.diMauro, L.Snyder, P.Marino, A.Lamberti, A.Coppo and G.P.Tocchini-Valentini, Nature, 222, 533 (1969).
57. A.A.Travers and R.R.Burgess, Nature, 222, 537 (1969).
58. Zh.G.Shmerling, Biochem.Biophys.Res.Commun., 37, 965 (1969).
59. (a) K.von der Helm and W.Zillig (Unpublished results); M.Rodriguez-Lopez, M.L.Munoz-Calvo and D.Vazquez (Unpublished results).
60. (a) W.Wehrli and M.Staehelin, Biochim.Biophys.Acta, 182, 24 (1969); (b) F.Knüsel, H.Bickel and W.Kump, Experientia, 25, 1207 (1969).
61. (a) H.E.Hemphill, H.R.Whiteley, L.R.Brown and R.H.Doi, Biochem,Biophys. Res.Commun., 37, 559 (1969); (b) R.Moss, E.Katz and E.N.Rosenblum, Biochem.Biophys.Res.Commun., 36, 858 (1969).

62. (a) Z.Ben-Ishai, E.Heller, N.Goldblum and Y.Becker, Nature, 224, 29 (1969); (b) Y.Becker, Y.Asher, N.Himmel, Z.Zakay-Rones and B.Maythar, Nature, 224, 33 (1969)
63. D.Vazquez, T.Staehelin, M.L.Celma, E.Battaner, R.Fernandez-Munoz and R.E.Monro, ref. 5, p. 100.
64. H.Masukawa, N.Tanaka and H.Umezawa, J.Antibiotics, 21, 73 (1968).
65. H.Masukawa, N.Tanaka and H.Umezawa, J. Antibiotics, 21, 517 (1968).
66. S.Sarkar and R.E.Thach, Proc.Nat.Acad.Sci.U.S., 60, 1479 (1968).
67. (a) J.A.Last, Biochim.Biophys.Acta, 195, 506 (1969); (b) J.A.Last, J. Izaki and J.F.Snell, Biochim.Biophys.Acta, 174, 400 (1969).
68. L.Luzzatto, D.Apirion and D.Schlessinger, Proc.Nat.Acad.Sci.U.S., 60, 873 (1968).
69. L.Luzzatto, D.Apirion and D.Schlessinger, J.Bact., 99, 206 (1969).
70. L.Luzzatto, D.Apirion and D.Schlessinger, J.Mol.Biol., 42, 315 (1969).
71. J.Modolell and B.D.Davis, Proc.Nat.Acad.Sci,U.S., 61, 1279 (1968).
72. J.Modolell and B.D.Davis, Nature, 224, 345 (1969).
73. R.Rosset and L.Gorini, J.Mol.Biol., 39, 95 (1969).
74. M.Nomura, S.Mizushima, M.Ozaki, P.Traub and C.V.Lowry, XXXIV Cold Spring Harb. Symp. Quant. Biol. (1969).
75. P.Traub and M.Nomura, Science, 160, 198 (1968).
76. H.Kaji and Y.Tanaka, J.Mol.Biol., 32, 221 (1968).
77. Y.Tanaka and H.Kaji, Biochem.Biophys.Res.Commun., 32, 313 (1968).
78. M.Ozaki, S.Mizushima and M.Nomura, Nature, 222, 333 (1969).
79. (a) A.Bollen, J.Davies, M.Ozaki and S.Mizushima, Science, 165, 85 (1969); (b) A.Bollen and A.Herzog, FEBS Letters, 6, 69 (1970).
80. L.Luzzatto, D.Schlessinger and D.Apirion, Science, 161, 478 (1968).
81. D.Apirion, D.Schlessinger, S.Phillips and P.Sypherd, J.Mol.Biol., 43, 327 (1969).
82. D.Apirion and D.Schlessinger, J.Bact., 96, 768 (1968).
83. P.F.Sparling, J.Modolell, Y.Takeda and B.D.Davis, J.Mol.Biol., 37, 407 (1968).
84. P.Anderson, J.Bact., 100, 939 (1969).
85. D.Apirion and D.Schlessinger, J.Bact., 96, 1431 (1968).
86. D.Apirion and D.Schlessinger, Proc.Nat.Acad.Sci.U.S., 63, 794 (1969).
87. (a) C.Guthrie, H.Nashimoto and M.Nomura, XXXIV Cold Spring Harb. Symp. Quant. Biol. (1969); (b) P.F.Sparling, Science, 167, 56 (1970).
88. (a) L.B.Cohen, A.E.Herner and I.H.Goldberg, Biochemistry, 8, 1312 (1969); (b) L.B.Cohen, I.H.Goldberg and A.E.Herner, Biochemistry, 8, 1327 (1969).
89. (a) F.N.Chang, C.Siddhikol and B.Weisblum, 186, 396 (1969); (b) C. Cocito, J.Gen.Microbiol., 57, 179 (1969); (c) S.Pestka, Archiv.Biochem. Biophys., 136, 80 (1970); (d) S.Pestka, Archiv.Biochem.Biophys., 136, 89 (1970).
90. (a) J.Cerna, I.Rychlik and P.Pulkrabek, European J.Biochem., 9, 27 (1969); (b) M.Yukioka and S.Morisawa, J.Biochemistry, 66, 241 (1969).
91. (a) A.E.Herner, I.H.Goldberg and L.B.Cohen, Biochemistry, 8, 1335 (1969); (b) R.E.Munro, M.L.Celma and D.Vazquez, Nature, 222, 356 (1969).
92. S.Pestka, Biochem.Biophys.Res.Commun., 36, 589 (1969).
93. S.Pestka, Proc.Nat.Acad.Sci.U.S., 64, 709 (1969).
94. R.E.Munro, R.Fernandez-Munoz, M.L.Celma, A.Jimenez, E.Battaner and D. Vazquez, Proc.Sixth Intern.Congress Chemotherapy, Tokyo (1969).

95. M.L.Celma, R.E.Monro and D.Vazquez, FEBS Letters, 6, 273 (1970).
96. (a) N.L.Oleinick and J.W.Corcoran, J.Biol.Chem., 244, 727 (1969); (b) J.C.H.Mao and M.Putterman, J.Mol.Biol., 44, 347 (1969); (c) K. Igarashi, H.Ishitsuka and A.Kaji, Biochem.Biophys.Res.Commun., 37, 499 (1969).
97. (a) D.Vazquez, E.Battaner, R.Neth, G.Heller and R.E.Monro, XXXIV Cold Spring Harb.Symp. Quant. Biol. (1969); (b) R. Neth, R.E.Monro, G.Heller, E.Battaner and D.Vazquez, FEBS Letters, 6, 198 (1970).
98. (a) H.Küntzel, Nature, 222, 142 (1969); (b) F.C.Firkin and A.W. Linnane, FEBS Letters, 2, 330 (1969); (c) W.Neupert, W.Sebald, A.J. Schwab, P.Massinger and Th.Bücher, European J.Biochem., 10, 589 (1969).
99. (a) C.Gurgo, D.Apirion and D.Schlessinger, J.Mol.Biol., 45, 205 (1969); (b) M.J.Weber and J.A.DeMoss, J.Bact., 97, 1099 (1969); (c) L.A. Phillips, B.Hotham-Inglewski and R.M.Franklin, J.Mol.Biol., 45, 23 (1969); (d) E.Cundlife, Biochemistry, 8, 2063 (1969).
100. N.Tanaka, T.Kinoshita and H.Masukawa, Biochem.Biophys.Res.Commun., 30, 278 (1968).
101. (a) M.Malkin and F.Lipmann, Science, 164, 71 (1969); (b) N.Tanaka, T. Nishimura, T.Kinoshita and H.Umezawa, J.Antibiotics, 22, 181 (1969); (c) J.W.Bodley, F.J.Zieve, L.Lin and S.T.Zieve, Biochem.Biophys.Res. Commun., 37, 437 (1969); (d) W.McKeehan and B.Hardesty, Biochem. Biophys.Res.Commun., 36, 625 (1969); (e) B.S.Baliga, A.W.Pronezuk and H.N.Munro, J.Biol.Chem., 244, 4480 (1969).
102. (a) S.Mizuno, K.Nitta and H.Umezawa, J.Biochemistry, 61, 373 (1967); (b) T.Nishimura, J.Antibiotics, 21, 110 (1968).
103. (a) Y.C.Lin, T.Kinoshita and N.Tanaka, J.Antibiotics, 21, 471 (1968); (b) F.Reusser, Biochemistry, 8, 3303 (1969); (c) D.Smithers, L.L. Bennett and R.F.Struck, Mol.Pharmacol., 5, 433 (1969).
104. T.J.Franklin and J.M.Cook, Biochem.J., 113, 515 (1969).
105. A.Tavitian, S.C.Uretsky and G.Acs, Biochim.Biophys.Acta, 157, 33 (1968).
106. C.T.Hardesty, N.A.Chaney, V.S.Waravdeker and J.A.R.Mead, Biochim. Biophys.Acta, 105, 579 (1969).

Section IV - Metabolic Diseases and Endocrine Function

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Chapter 16. Prostaglandins and Related Compounds

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"In Atlanta, volunteers are inhaling a hormone-like substance to clear up Nasal congestion. In Stockholm it is being tested against male sterility. In Uganda, it is injected to induce labor. And in Michigan, researchers are feverishly exploring its possibilities as a "morning after" contraceptive."

Medical World News, February 7, 1969

The above statements vividly describe some of the areas in which clinicians are attempting to put prostaglandins (PG) to therapeutic use. Since the publication of the last report[1] in this series, many reviews have appeared of which the one by E. W. Horton is particularly worthy of mention. The article[2] explicitly brings out the biochemical and pharmacological profiles of prostaglandins and points out possible lines for future investigation. Nasal decongestion, lowering blood pressure, treating gastric ulcers, treating infertile semen, inducing labor and treating thrombosis are some of the promising therapeutic areas that are being investigated for prostaglandins. Necessary prerequisites for such studies are adequate supplies. This has greatly motivated research on the chemistry of prostaglandins.

Because the biology, but not the chemistry, of these substances has been well covered in the recent reviews, the present report will be limited to discussion of extensive recent chemical advances.

The report will be divided into two sections (a) Syntheses of natural prostaglandins and (b) Syntheses of unnatural prostanoic acids and related analogs. Since PGEs can be transformed to PGFs and PGAs, any synthesis of PGE also constitutes, in principal, a synthesis of the corresponding PGF and PGA*.

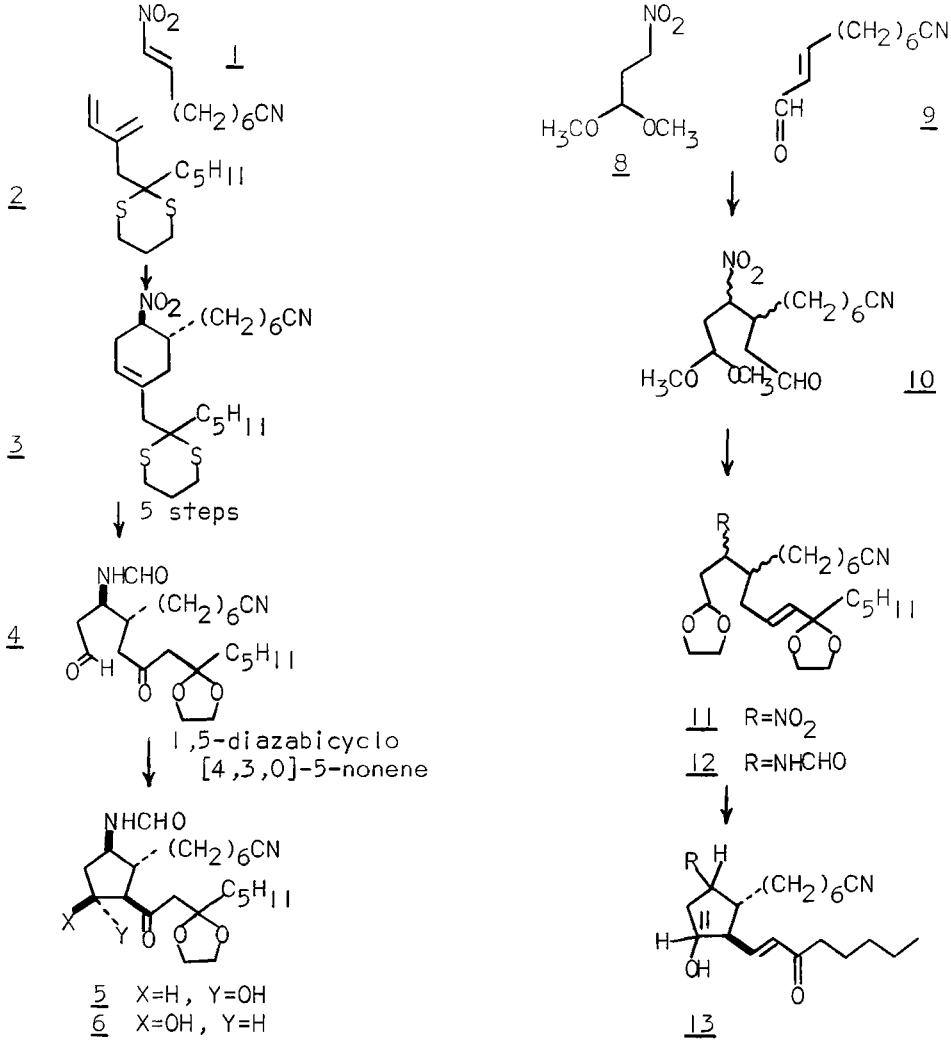
(a) Syntheses of natural prostaglandins - The syntheses of PGE_1 and PGE_2 in their racemic and that of PGE_1 in its optically active form constitute notable achievements by E. J. Corey and his associates.[3] Their synthetic sequences are particularly characterized by mild and specific reaction conditions. The first two syntheses of PGE_1 from this group basically involved an intramolecular aldol condensation to generate the desired cyclopentanol, followed by transformation[3c] of an amino group at C-9 to a ketone function.

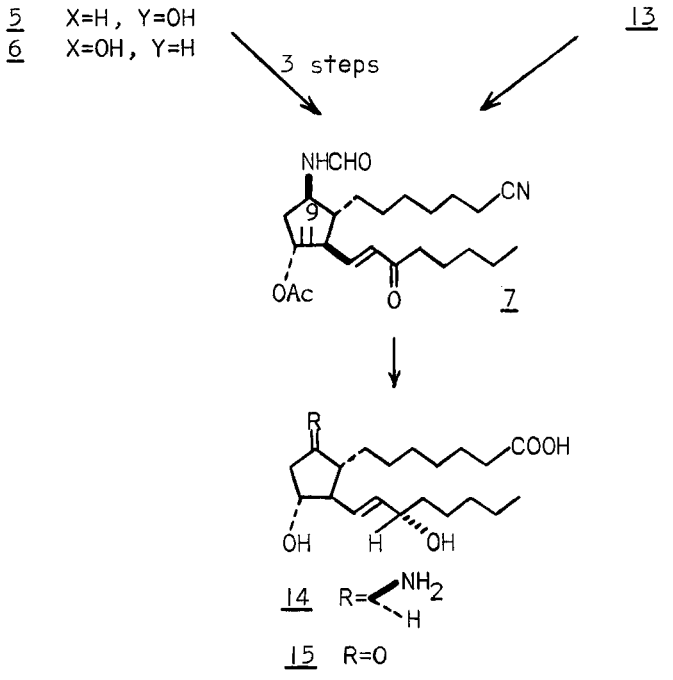
* For details of nomenclature see ref. 2.

The substrate 4 required for cyclization was obtained [3a] from the Diels-Alder adduct 3 (derivable from 1 and 2) in five steps involving i) reduction ii) formylation iii) thioketal --> ketal exchange iv) osmylation and v) cleavage of vic-glycol.

In another sequence[3b] 3-nitro propanal dimethyl acetal 8 was condensed in Michael manner with 9-cyano-2-nonenal 9 to yield aldehyde 10, which was transformed in two steps to a desired substrate 11 for intramolecular ring closure.

The cyclization of 4 yielded the alcohol 5 in 45% yield as its acetate with some epimeric alcohol 6. This was transformed in three steps to enone 7.

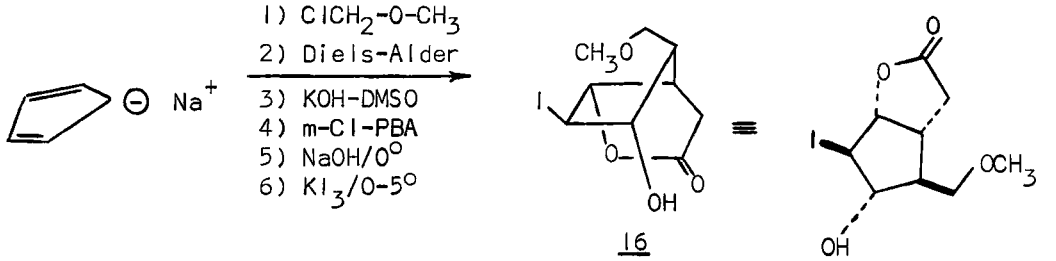


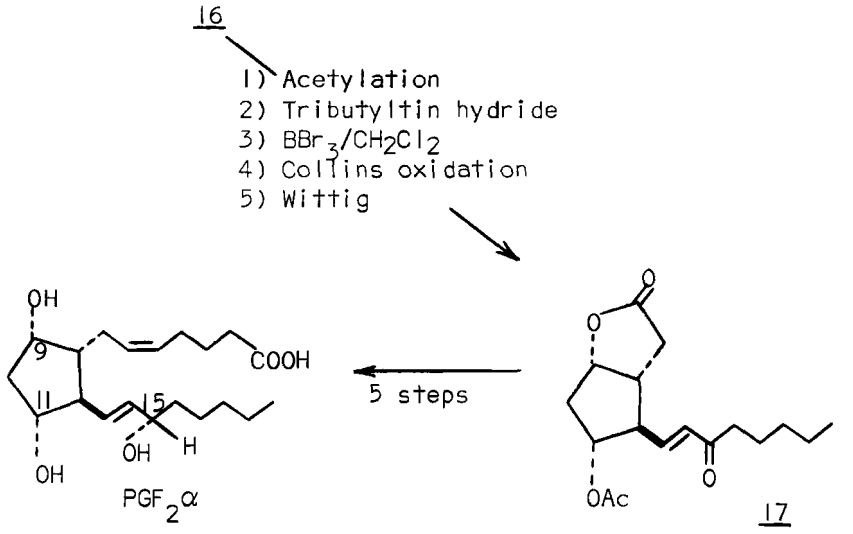


The enone 7 was also obtained in an alternative sequence as follows: Cyclization of dioxolane 12 in acetone-p-toluensuiphonic acid led to a mixture of four possible stereoisomeric alcohols 13, from which enone 7 could be isolated after acetylation. The ratio of the pairs of C-11 epimers formed in cyclization was found to be dependent on the conditions employed. Thus, cyclization[3d] of 11 with stannic chloride in acetone gave predominantly the desired C-11α alcohols.

αβ-Unsaturated ketone 7 was transformed via i) reduction ii) deacetylation iii) THP ether iv) hydrolysis (CN--> COOH and NHCHO-->NH_2) to yield the amino acid 14. Dehydrobromination of the N-bromo derivative followed by hydrolysis at pH2 generated the PGE_1 15.

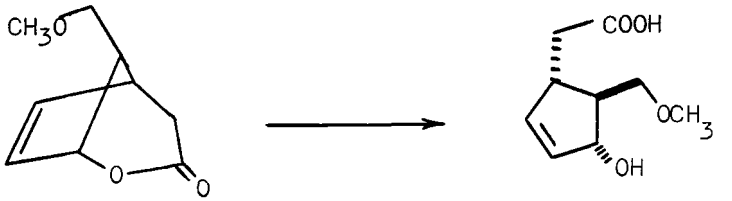
A more recent stereoselective approach[3c] by the Harvard group led to the synthesis of PGE_2 and $PGF_{2\alpha}$.





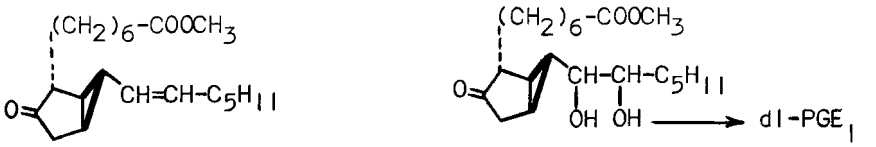
The penta substituted cyclopentane 16 was synthesized by a six step stereoselective sequence starting with cyclopentadienyl sodium. Lactone 16 was transformed in five steps to α,β-unsaturated ketone 17. This was converted in five more steps to $PGF_{2\alpha}$. A sequence was developed whereby C-11 and C-15 alcohols can be protected as THP ethers, and the C-9 ketone generated by oxidation leading to PGE_2.

Resolution of intermediate acid 17b obtained from hydrolysis of 17a was achieved with (+)-ephedrine in 67% yield. The synthesis described

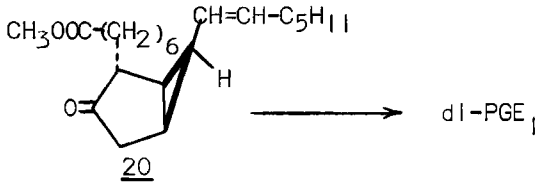


above was carried through with optically active acid to complete preparation of optically active $PGF_{2\alpha}$ and PGE_2[19].

Another group engaged actively in the chemistry of prostaglandins, has been able to resolve[4] some of the discrepancies reported earlier[1]. Four isomeric diols 19[4b] were isolated in pure form by hydroxylation of



olefins 18 with osmium tetroxide. Bismesylation followed by solvolysis led to the isolation of dl-PGE_1 methyl ester in 4-8% yield. In contrast to the exo series, the same sequence of reactions, when applied to endo



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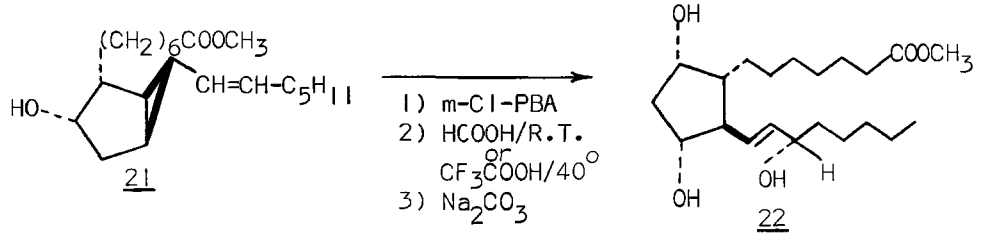
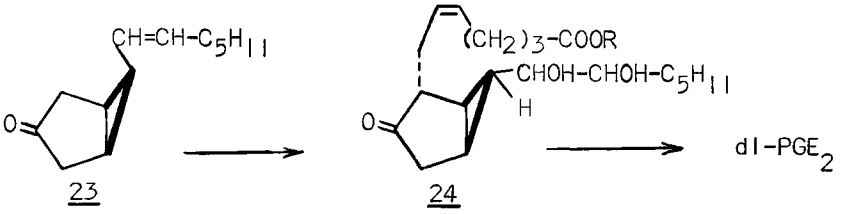
olefin 20,[4d] produced PGE_1 in 17-18% yield. An independent synthesis[4a] of $PGF_{1\alpha}$ was also carried out as follows: The olefin 21 was epoxidized with m-chloroperbenzoic acid. Acid treatment of the resulting epoxide led to isolation of $PGF_{1\alpha}$ methyl ester 22 in 2.5% yield.



21 22

The same general approach was utilized to synthesize[4c] PGE_2 and $PGF_{2\alpha}$.

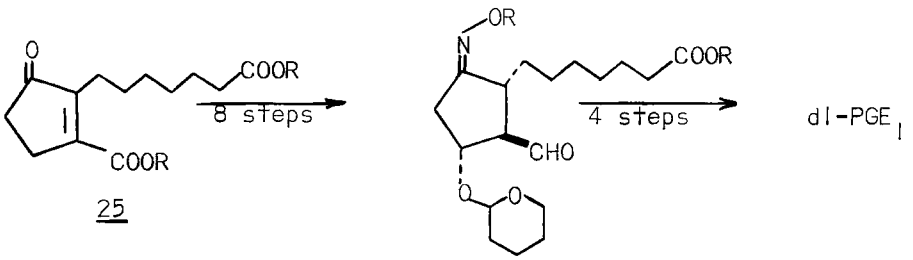


23 24

Ketone 23 was converted in eight conventional steps to diol 24. This diol, upon bismesylation followed by solvolysis, led to dl-PGE_2 in about 15% yield.

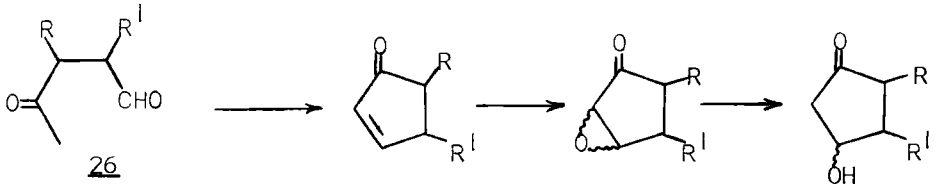
A recent article[16] from the Upjohn group has described various preparative scale transformations of natural prostaglandins. Particularly noteworthy among them is the formation of 15-epi compounds on treatment of natural 15-(S) products with formic acid-sodium formate at room temperature.

Another synthetic approach to PGE_1 was reported by the Ciba group[16]. This utilizes as its starting point the cyclopentenone 25, obtained in eight steps from readily available starting materials. Compound 25 is converted in twelve steps to PGE_1. Use of trisilyl ether and oxime protecting groups for C-11 hydroxyl and C-9 ketone respectively, are noteworthy.

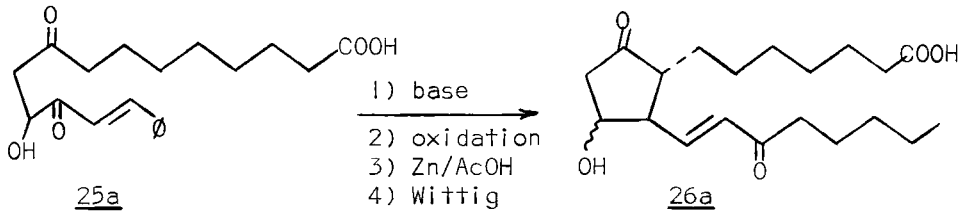


It should be noted that both the Harvard group and the Upjohn group in the course of their synthetic work have described the epimeric and isomeric prostaglandins. Thus, racemic 11-epi, 15-epi, and 11,15-diepiprostaglandins were isolated by the former group, whereas racemic 8-iso, and 15-epi prostaglandins were reported by the latter group.

(b) Syntheses of unnatural prostanoic acids and related analogs - A synthetic approach to dihydro PGE_1 has appeared in the patent literature[17]. The stereochemistry of the substituents was not defined. The patent elaborates a series of reactions, starting with acyclic chemicals, to arrive at substituted levulinic aldehyde 26. This was then cyclized and transformed as shown to a substituted cyclopentanone having the correct substituents of 13,14-dihydro PGE_1.

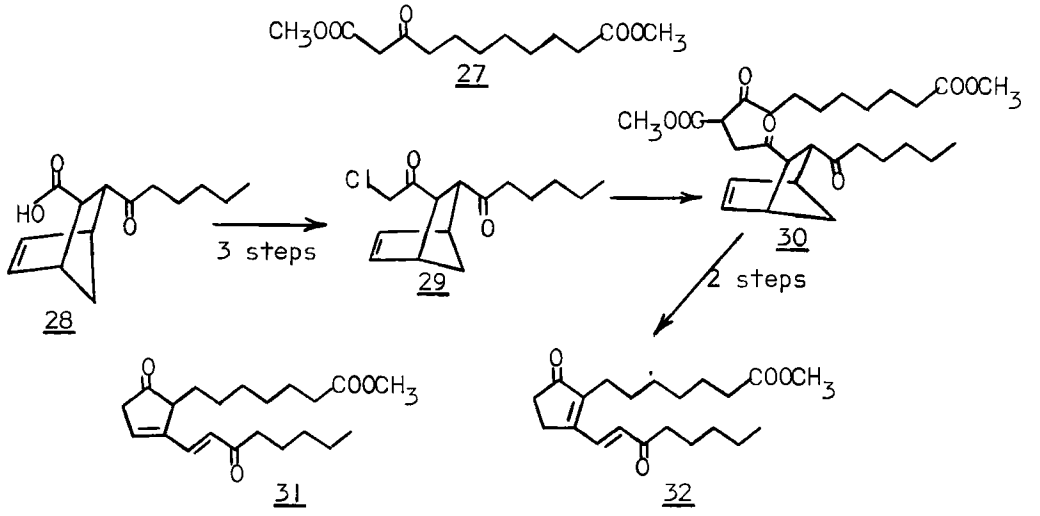


A synthesis of dl-15-dehydroprostaglandin E_1 was reported by Miyano[5]. The starting acid 25a was obtained from condensation of 3-ketoundecan-1, 11-dioic acid and styryl glyoxal with concomitant decarboxylation. Intramolecular ring closure followed by oxidative fragmentation of the styryl

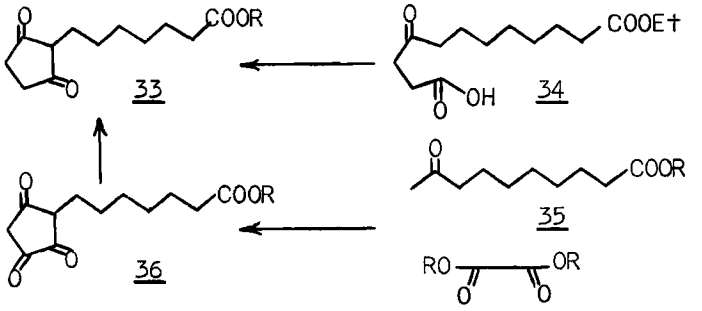


double bond, zinc-acetic acid reduction and a Wittig reaction with 2-oxoheptyl triphenyl phosphorane yielded compound 26a in 12-15% yield (from 25a) as a mixture of C-11 epimeric alcohols. The same author also reported the synthesis[6] of 15-dehydro PGB_1. The main feature of this synthesis is the use of the bicyclo [2,2,1]-hept-5-ene system as a protecting

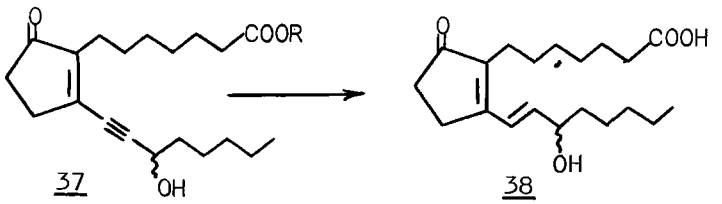
group for a double bond. Acid 28 was transformed by a conventional route to diketone 29. Condensation of dimethyl 3-ketoundecandioate 27 with diketone 29 led to compound 30. This underwent intramolecular condensation



followed by pyrolytic removal of cyclopentadiene in a reverse Diels-Alder reaction to generate 15-dehydro PGB_1 32. The dienone 31 was a by-product in the synthesis of 15-dehydro PGE_1 26a. However, a total synthesis of diketone 31 has also been reported.[7] Three different groups reported the synthesis of PGB_1 (PGE_1-278).[8] All of these used the diketone 33 as their starting substrate. One group[8] synthesized diketone 33 by cyclization of

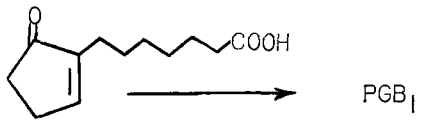


monoester acid 34 in presence of aluminum chloride and propionyl chloride. The other two groups[9,10] followed an established sequence, namely the condensation of 9-oxodecanoic acid (or ester) 35, with oxalic acid ester (R= CH_3 or C_2H_5), followed by treatment with acid to yield triketone 36. This was converted under reductive conditions to diketone 33. Ketone 33 was transformed via i) enol-ether, ii) reaction of vinylogous ester with appropriate alkynyl Grignard and iii) acid hydrolysis to produce the keto alcohol 37. This was converted by reduction (triple bond to double bond) to PGB_1 38. The Unilever group[11] reported a synthesis of PGB_1 starting



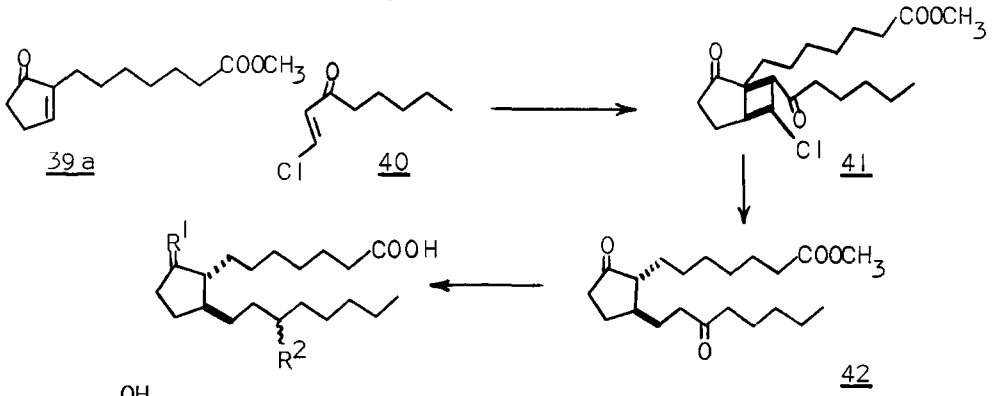
37 38

with unsaturated ketone 39 reported earlier by the Ayerst group[12] and following a sequence essentially similar to that described by Hardegger and coworkers.[12]



39 38

A novel approach to prostanoic acid was reported from Ayerst Laboratories.[13] Irradiation of unsaturated keto ester 39a and 1-chloro-3-oxooct-1-ene 40 led to the formation of bicyclic diketone 41. Treatment of the photoadduct with zinc and acetic acid led to the formation of methyl 9,15-dioxoprostanoate 42. This was transformed under varying reductive conditions to compounds 43 - 47, all of which showed prostaglandin-like activities in different pharmacological tests.



39a 40 41 42

43 R^1 = , R^2=OH

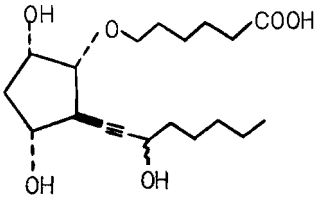
44 R^1 = , R^2=OH

45 R^1 = , R^2=OH

46 R^1 = , R^2=

47 R^1 = , R^2 =

Fried[14] and coworkers reported the synthesis of some 7-oxa analogs of prostanoic acid and their cyclohexane analogs. Some of these compounds were found to be prostaglandin antagonists. Compounds 48 and 49 have also been shown[14b] to produce smooth muscle responses.



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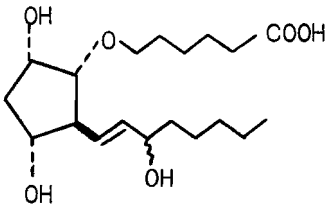
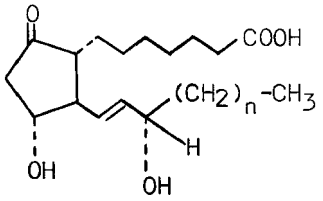


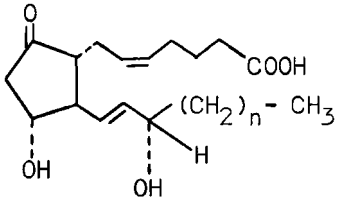
49

The Unilever group[15] has reported the biological conversion of 19-and 21-carbon fatty acids with 3 and 4 double bonds to produce ω-nor-PGE_1 50 and $-PGE_2$ 51; and ω-homo PGE_1 52 and $-PGE_2$ 53 respectively. The rates of formation are slow, however, relative to the 20 carbon acids. The nor- and homo-prostaglandins did exhibit prostaglandin-like activity.



50 n=3

52 n=5



51 n=3

53 n=5

Finally, a biochemical synthetic procedure[18] for C-18 and C-19 oxygenated prostaglandins has also been reported. Starting with 5,8,11,14-eicosatetraenoic acid and exposing it to Ophiobolus graminis yields 18ε- and 19ε-hydroxy derivatives. These were cyclized to the corresponding prostaglandins by bull seminal vesicle microsomes.

In conclusion, natural prostaglandins have been synthesized, which in principle provides unambiguous proof of their structure. Biological activities of various isomers and epimers have been reported. Knowledge of structural requirements for activity has also been augmented by synthesis and evaluation of some synthetic analogs. The question as to whether chemically derived prostaglandins and related compounds offer advantages over naturally produced materials is yet to be answered.

Acknowledgement - The author gratefully acknowledges helpful discussions with Dr. T. Bogri on several points of interest.

References

1. U. Axen and E. E. Smissman, Annu. Rep. Med. Chem., 290 (1967).
2. E. W. Horton, Physiol. Rev. 49 (1), 122 (1969).
3.a)E. J. Corey, N. H. Anderson, R. M. Carlson, J. Paust, E. Vedejs, I. Vlattas and R. E. K. Winter, J. Amer. Chem. Soc. 90, 3245 (1968).
b)E. J. Corey, I. Vlattas, N. H. Anderson and K. Harding, ibid., 90, 3247 (1968).
c)E. J. Corey and K. Achiwa, ibid., 91, 1429 (1969).
d)E. J. Corey, I. Vlattas and K. Harding, ibid., 91, 535 (1969).
e)E. J. Corey, N. M. Weinshenker, T. K. Schaaf and W. Huber, ibid., 91, 5675 (1969).
f)P. W. Ramwell, J. E. Shaw, E. J. Corey and N. Anderson, Nature, (London) 221, 1251 (1969).
4.a)G. Just, C. Simonovitch, F. H. Lincoln, W. O. Schneider, U. Axen, G. B. Spero and J. E. Pike, J. Amer. Chem. Soc. 91, 5364 (1969).
b)W. P. Schneider, U. Axen, F. H. Lincoln, J. E. Pike and J. L.Thompson, ibid., 91, 5372 (1969).
c)E. G. Daniels, W. C. Kruger, F. P. Kupiecki, J. E. Pike and W. P. Schneider, ibid., 90, 5894 (1968).
d)W.P. Schneider, Chem. Commun. 304 (1969).
e)J. E. Pike, F. H. Lincoln and W. P. Schneider, J. Org. Chem., 34, 3552 (1969).
5. M. Miyano and C. R. Dorn, Tetrahedron Lett., 1615 (1969).
6. M. Miyano, ibid., 2771 (1969).
7. R. B. Morin, D. O. Spry, K. L. Hauser and R. A. Mueller, ibid., 6023 (1968).
8. Y. Yura and J. Ide, Chem. Pharm. Bull., 17, 408 (1969).
9. P. Collins, C. J. Jung and R. Pappo, Isr. J. Chem., 6, 839 (1968).
10. J. Katsube and M. Matsui, Agr. Biol. Chem., 33, 1078 (1969).
11. R. Klok, H. J. J. Pabon and D. A. Van Dorp.,Rec. Trav. Chim. Pays-Bas, 87, 813 (1968).
12. See ref. No's. 12 and 18 in U. Axen and E. E. Smissman, Annu. Rep. Med. Chem., 290 (1967).
13. J. F. Bagli and T. Bogri, Tetrahedron Lett., 1639 (1969).
14. a) J. Fried, T. S. Santhanakrishnan, J. Himizu, C. H. Lin, S. H. Ford, B. Rubin and E. O. Grigas, Nature (London) 223, 208 (1969).
b) S. H. Ford and J. Fried, Life Sci. 8, Part I, 983 (1969).
15. R. K. Beerthuis, D. H. Nugteren, H. J. J. Pabon and D. A. Van Dorp., Rec. Trav. Chim. Pays-Bas, 87, 461 (1968).
16. N. Finch and J. J. Fitt, Tetrahedron Lett., 4639 (1969).
17. Belgian Pat. No. 72,7755, American Home Products, Farmdoc No. 39601.
18. C. J. Sih, G. Ambrus, P. Foss, and C. J. Lai, J. Amer. Chem. Soc., 91, 3685 (1969).
19. E. J. Corey, T. K. Schaaf, W. Huber, U. Koelliker, N. M. Weinshenker, ibid., 92, 397 (1970).

Chapter 17. Atherosclerosis

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Division of Carter-Wallace, Inc., Cranbury, New Jersey

Introduction - Atherosclerosis is a complex and multifaceted condition which may be a collection of pathological states with overlapping similarities of arterial abnormalities. Although atherosclerosis occurs throughout the world, its greatest frequency is found among the "advanced" or affluent nations where, at the present time, atherosclerotic cardiovascular disease is the most common cause of death. These mortality statistics underscore the urgency for control of a disease whose pathogenesis is still only dimly understood.

Numerous disciplines have been brought to bear on the intricacies of atherosclerosis and its sequelae. While etiological, epidemiological, prophylactic and therapeutic studies have frequently received attention, increasing emphasis is being placed on multidiscipline investigations and research at the molecular level. The recent availability of more sophisticated techniques has materially complemented the efforts currently devoted to lipid metabolism and lipoprotein chemistry.

A number of reviews dealing with atherosclerosis were published in 1969[1-3], but the comprehensive book by Schlettler and Boyd[4] entitled "Atherosclerosis" should be a required reference for those in the field. The Second International Symposium on Atherosclerosis, held in Chicago in November, 1969, was noteworthy for the broad and varied approaches discussed. The publication of the proceedings should also be a handy reference in the field.

Etiology - The low incidence of atherosclerosis in individuals in the less technologically developed areas compared to the high incidence of this condition in residents of the technologically advanced countries suggests that culture and custom are related to prevalence of the disease. The importance of these differences in everyday living habits has been outlined in the studies of people who migrate from low incident areas to high incident areas. After the migrating group assimilates the new culture and adapts to the new environment, its incidence of atherosclerosis increases and becomes indistinguishable from other individuals in its acquired culture. Thus, it is not surprising that numerous factors have been identified as environmental risks. They include stress, smoking, lack of exercise, hypertension, serum lipid levels and most importantly diet. Other conditions predisposing to atherosclerosis that are less well identified as environmentally connected include genetic makeup, endocrine aberration, immunological

and autonomic factors, blood flow, blood coagulation and obesity.

While much has been learned concerning predisposing conditions, it is quite possible that elimination or severe reduction of atherosclerosis awaits more fundamental findings as to the mechanism of atheromata formation in situ. Plaque pathogenesis must occur at the molecular level in the vessel wall and very little is known regarding this phenomenon. There are, however, two prevalent concepts on the genesis of atheromatous plaques, the filtration theory and the thrombogenic theory. They involve different mechanisms and are not easily reconcilable. The filtration theory presumes that plasma constituents enter directly into the arterial wall by a process of diffusion from the lumenal surface of the vessel. Injury to the endothelium and/or intima or a high level of lipoprotein encourages plaque formation. In the other major theory, mural thrombi adhere to the lumenal surface and become incorporated into the wall by an overgrowth of endothelium. The type of plaque formed is dependent upon the ratio of the adhering materials, platelets and fibrin.

Lipid removal may be a critical factor[5] in plaque formation and the location of lecithin-cholesterol transacylase in the arterial wall[6] could play an important role since cholesterol is rapidly exchanged[7] with the blood while cholesterol ester is not. Kuo[8,9] in an editorial cites the evidence for the theory that a disturbance in carbohydrate metabolism can be responsible for atherosclerosis and should be included as one of the primary risk factors in coronary heart disease. In this regard, Clements and coworkers[10] have shown the presence in aorta of aldose reductase, an enzyme which they feel provides a mechanism for the alteration of arterial metabolism by hyperglycemia. Another approach to molecular interactions was described by Levy and Day[11] who concluded from their results that the low density lipoproteins are uniquely polycationic at the surface and that these ions react with the internal arterial macromolecular polyanions.

As techniques become more sophisticated, more attention is being directed toward the lipoproteins. Stein and Stein[12] concluded from their own and other studies that lipoprotein release is regulated by the rate of fatty acid synthesis and esterification. Lees has reported that protein portions of the lipoproteins may be critical in atherogenesis[13] while Slack and Mills[14] indicate that in familial hyperbetalipoproteinemia an abnormal lipoprotein is present. Several authors have reported that serum globulins may play a role in hyperlipemia in individual patients either by their absence[15] or by complexing with a lipoprotein[16]. Platelets and thrombus formation which undoubtedly play a role in atheromata

formation were discussed and summarized at the International Symposium on Atherosclerosis[17-21].

Two environmental factors receiving attention during 1969 were the degree of hardness of water and hypoxia. A number of publications have supported the theory that the lack of oxygen can cause atheromata formation. Kjeldsen and co-workers could reverse rabbit atheromata by subjecting the animals to 28% oxygen[22] or could form atheromata by exposing the rabbits to carbon monoxide[23]. They found a correlation of the levels of carboxyhemoglobin in smokers with a diagnosis of arteriosclerotic vascular disease[24]. Garbarsch et al.[25,26] also found increased pathological arterial changes in rabbits induced by hypoxia. While a number of studies have supported the concept that cations, particularly those found in hard water, are beneficial in atherosclerosis[27,28], Filo et al.[29] found that zinc therapy enhanced atheromata formation. Schroeder[30] on the other hand found that chromium protected glucose-fed rats from developing hypercholesteremia.

In a preliminary report, Stamler and coworkers[31] described their findings that high resting heart rate is an important risk factor for coronary mortality. Their conclusion was reached in a study which involved 1329 male employees of a Chicago utility company over a ten-year period.

Exercise was reported to increase cholesterol degradation, as measured by respiratory excretion of $^{14}CO_2$, in healthy male patients receiving 26-^{14}C cholesterol[32]. Nestel et al.[33] concluded from their study of cholesterol distribution and turnover in humans that inadequate excretion of the sterol could be of importance in the development of hypercholesteremia, while Kotte[34] found differences in bile acid excretion in patients having either primary hypercholesteremia or combined hypercholesteremia and hypertriglyceridemia. The latter group excreted almost four times the bile acid of the former patients.

Stress or psycho-social factors have often been described as conducive to atherosclerosis disease. This approach to the causation of myocardial injury is discussed in a provocative article by Roab[35]. It is also interesting to note that Friedman and coworkers[36] were able to induce hypercholesteremia in the rat neurogenically by an electrolytic lesion in the brain.

The role of cholesterol absorption, synthesis and turnover in disease states and under therapeutic conditions has been given new stimulation by the publications of Grundy and Ahrens[37,38]. These authors described their isotope kinetic and sterol balance technique which enables them to measure the

effects of various therapeutic regimens on these cholesterol parameters.

Therapy - The treatment of atherosclerosis, although somewhat empirical, has advanced steadily over the past few years aided materially by the developments of (1) phenotyping of hyperlipoproteinemia, (2) accumulation of reliable information on various dietary regimens and (3) the availability of hypolipemic drugs. Thus, Levy and coworkers[39] have been able to report that the treatment of familial hypercholesteremia has shifted from a frustrating to an eminently treatable problem.

Diet is one of the most important factors in the prophylaxis of atherosclerosis. After the disease has manifested itself, diet control is usually a mandatory part of the therapeutic regimen. Generally, diets are directed toward the reduction of obesity and replacement of meat and saturated fat products with foods containing unsaturated fats and non-meat high protein substances[40].

Connor et al.[41], utilizing the sterol balance technique, found that corn oil not only had a hypocholesteremic effect but also induced excretion of cholesterol from the tissues. This effect was not observed with the more saturated fat, cocoa butter. Spritz and Meshkel[42] postulate a physical mechanism for the lipid lowering effect of unsaturated dietary fat. They suggest that the unsaturated fatty acids occupy a greater area than the saturated fatty acids and thus alter the spatial configurations of the lipids into which they are incorporated. As a result, fewer lipid molecules can be accommodated by the apoprotein moiety of the low density lipoprotein.

Chemotherapy of atherosclerosis (not including adjuvant treatment such as anticoagulants and hypotensives) is achieving an increasingly substantial role in the treatment of the disease. Drug use has centered on compounds or substances that will lower specific serum lipids, notably cholesterol and/or triglycerides. In addition to those already available commercially (dextrothyroxine, clofibrate, β-sitosterol, estrogens, nicotinic acid, cholestyramine, etc.), a number of new and old compounds were extolled during 1969 for their antiatherogenic properties. A plethora of literature on clofibrate (CPIB), 2-(p-chlorophenoxy)-2-methylpropionic acid ethyl ester, includes several reports citing its activity in Type-II hyperlipoproteinemia[43,44] although others have found it to be less potent in this area[45]. CPIB was found to demonstrate activity to Types III, IV and V hyperlipoproteinemia[46]. Of greater significance perhaps than the serum lipid reduction is the report by Albert and Stansell[47] who found that, in addition to its hypocholesteremic effect,

clofibrate regressed skin xanthomas, relieved vertigo in patients with cerebrovascular ischemia and increased exercise tolerance in a group with peripheral atherosclerosis. In patients with primary biliary cirrhosis, however, clofibrate elevated serum cholesterol and increased the severity of xanthomas[48]. Kohatnur et al.[49] and Zakim and Herman[50] found that the hypolipidemic effect of CPIB is dependent upon the nature and type of diet. Further exploration of postulated mechanisms of action of clofibrate are found in the reports of Cenedella et al.[51] and Ruegamer and coworkers[52]. The former workers found that CPIB lowered plasma and reduced free fatty acids (FFA) and hence reduced FFA availability while the latter experimenters observed that CPIB caused a displacement of thyroxine from the plasma into the liver. McKerron and his collaborators[53], however, reported that clofibrate did not have significant effects on thyroxine.

Preliminary evidence reported by several groups[54,55] have indicated that administration of clofibrate induces a depletion of body sterol by increasing sterol excretion without affecting or lowering the rate of cholesterol biosynthesis. Verification of these findings and application of the techniques employed to other hypolipidemic agents should provide some of the more interesting results during 1970.

Activity of a number of clofibrate analogues was described for use in the treatment of atherosclerosis. Best and Duncan[56], Hartmann and Forster[57], Berkowitz[58], and Mancini et al.[59,60] reported studies in man of Su-13437, 2-methyl-2-[p-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxy]-propionic acid. The most extensive study was that of Hartmann and Forster[57] who found that in 88 patients Su-13437 was effective in reducing the serum triglyceride and cholesterol levels of Types III, IV and V hyperlipidemia while the Type II patients responded to a lesser degree. They concluded, as did the other authors[56-58], that Su-13437 was more potent than clofibrate, particularly with Type II individuals and that like CPIB it possesses greater hypotriglyceridemic than hypocholesteremic activity. In preliminary findings, Berkowitz[58] reported that SaH 42-348, 1-methyl-4-piperidyl bis(p-chlorophenoxy)acetate was effective in reducing serum triglyceride and serum cholesterol. Timms et al.[61] who worked with rats also found that this clofibrate analogue was a more active hypolipidemic agent than the parent compound.

A summary of the clinical studies carried out with dextrothyroxine (D-T4) was prepared by Bechtol and Warner[62]. They analyzed data obtained from 6066 patients and concluded that this drug is effective in lowering elevated serum cholesterol in both hypothyroid and euthyroid patients although the effect is somewhat greater in the hypothyroid individual.

Cohen[63] in an eight-year appraisal of clinical use of dextrothyroxine concluded that D-T4 is an effective agent in lowering blood cholesterol. He cautioned, however, against its use in patients with coronary heart disease. A comparative study with clofibrate, dextrothyroxine and niacin indicated that all three compounds lowered serum cholesterol[64].

Limitation of cholesterol absorption either by removal of bile acids or by competitive inhibition continued to be investigated. Cholestyramine was found to consistently reduce the LDL cholesterol with a possible offsetting of this action by a slight rise in the VLDL cholesterol[65]. Over-all, cholestyramine had a definite hypocholesteremic action. A new copolymer of tetraethylenepentamine and epichlorohydrin, U-26597A, may be somewhat more effective than cholestyramine[66]. The mechanism for the decreased cholesterol absorption following neomycin administration was reported to be due to the drug's ability to increase bile acid secretion as well as its known alteration of intestinal flora[67]. The inhibition of cholesterol esterification was suggested as the mechanism for the cholesterol absorption reduction induced by cholestane-3β,5α,6β triol[68].

A new compound, N-γ-phenylpropyl-N-benzyloxy acetamide (W-1372), was described in several publications by Berger and coworkers[69,70]. These authors found that W-1372 lowered blood cholesterol, phospholipids and triglycerides in two species of monkeys on a normal diet but not in similarly treated rats and rabbits. The drug was effective in rats on hypercholesteremic diet and perhaps more importantly reduced the extent of fatty deposits in the aorta of squirrel monkeys and rabbits. The latter observation was confirmed in Kritchevsky's laboratory[71]. The mechanism of action of W-1372 is probably different from that of clofibrate since these drugs produce different effects in various experimental regimens[70]. DH-581, /4,4'-(isopropylidenedithio)bis(2,6-di-t-butylphenol)/ was reported by Drake et al.[72] to be effective in lowering serum cholesterol and phospholipid in a limited number of subjects. The effect of this compound on triglyceride levels awaits more substantial data for evaluation although Barnhart et al.[73] found it an effective hypotriglyceridemic agent in rats.

Pyridinolcarbamate, a compound known for some time, was found by Atsumi[74] to dissolve cholesterol deposits in arterial walls and also to promote regeneration of damaged arteries. It would be interesting to get more details and confirmation of this preliminary report. Wu et al.[75] presented evidence that pyridinolcarbamate was effective in preventing aortic fatty streak involvement in rabbits maintained on a high cholesterol diet but did not alter the

serum cholesterol level of these animals. A ferrocene derivative, N-(ferrocenylmethyl)piperidine, was reported to be a hypocholesteremic compound which altered the metabolism of 7-dehydrocholesterol[78]. Further evidence of the effectiveness of DL-N-(α-methylbenzyl)linoleamide as an antiatherosclerotic agent was presented by Nakatani and coworkers[77-79]. Since the D isomer was found to be more potent than the DL mixture, it would be of interest to see this purified isomer tested in systems other than the rat and rabbit.

Additional substances of interest which were cited in the literature for their effect in atherosclerosis or related conditions include β-benzal butyric acid[80], 2-[p-(2-diethylaminoethoxy)phenyl]benzimidazole and its naphthyl analogue[81], 1,2-α-oxido-4,6-androstadiene-3,17-dione[82], 2,2'''[(1-methyl-4,4-diphenylbutylidene)-bis(p-phenyleneoxy)]bistrimethylamine (SQ 10591)[83], chlorpheniramine[84], chlorcyclizine[85], 2-ethyl-n-caproic acid[86], glutamic acid[87], nicotonic acid and 3-methylpyrazole-5-carboxylic acid[88], 2(R),3(R)-dihydroxy-4-(9-adenyl)-butyric acid designated either lentinacin[89] or lentysine[90], dextran 40[91], chondroitin sulfate[92], and tomato pectin[93]. Compound series studied include 5-substituted tetrazoles[94] and derivatives of 2-(p-chlorophenoxy)-2-methylpropionic acid and 2-(p-chlorophenylthio)-2-methylpropionic acid[95].

In evaluating therapeutic approaches, one should not overlook the effort that is being expended on the surgical approaches such as ileal bypass[96] and vasodilation of the small arteries[97]. New techniques which show promise for early diagnosis and evaluation of therapeutic procedure are the adaptation of ultrasound[98,99] and bioelectric impedance[100] to arterial flow measurement.

Long-term Studies - Any therapeutic approach cannot be considered successful if it only lowers serum lipids. It must prevent further deposition in the tissues and hopefully also reduce existing atheromata. The final criterion of any treatment is its effect on the incidence of clinical vascular damage and useful life span. It has long been recognized that the efficacy of medical approaches to atherosclerosis can best be evaluated in long-term studies.

There are in progress two significant dietary trials for the primary prevention of atherosclerosis. They are the Anti-Coronary Club in New York and the Mental Hospital Study in Helsinki. In a summary report of the first study, Christakis and Rinzler[40] report that their dietary regimen evaluated over periods as long as five years was effective in lowering serum cholesterol levels, obesity and hypertension. Most importantly, however, the dietary group achieved a significantly lower incidence of morbidity from coronary heart disease than

the comparable control group. Similar findings were noted by Turpeinen in a brief report on the Helsinki study[101].

Two systematic studies of a number of drugs with appropriate double blind designs are underway in 53 cooperating centers in the United States and Great Britain. The Veterans Administration multicenter drug lipid cardiology trial is evaluating estrogens, aluminum nicotinate and dextrothyroxine while the British study is studying clofibrate[102-104]. These studies which include various dosage levels and drug combinations are aimed at evaluating the long-term effects of drug administration on morbidity in patients with a prior history of clinical heart disease. Altogether some 10,000 men, ages 30-60, will be participating over a five-year period.

Several authors[105,106] have commented that a long-term prevention of athrosclerosis could be practical if initiated at a pediatric age, since at that time, although fatty streaks are present, plaques have not formed and could be prevented by appropriate dietary and other measures.

A report of the Framingham study now in its 14th year and soon to be limited by government funding cutbacks, discusses the findings regarding serum lipid fractions and coronary heart disease[107]. The authors, Kannel, Costelli and McNamara, conclude that blood lipid content is related to atheromatous deposition, but many other factors also exert a marked influence on the disease. The study was unable to answer the question as to whether the hyperlipidemia was caused by genetic inability to handle a lipid diet or was due to prolonged overloading of normal metabolic functions. Collens[108,109] proposes that man is anthropologically a herbivore and hence cannot manage a lipid-rich carnivorous diet. He cites as evidence the practical disappearance of coronary thrombosis in countries deprived of meat products during war time, epidemiological studies comparing vegetarian to non-vegetarian diets and relative anatomical features of herbivores and carnivores.

Critics of this theory would undoubtedly cite the information gathered about the Masai of Africa who subsist on a diet rich in animal fat and yet have low serum lipid levels and little atherosclerotic disease. Recent findings[110], however, suggest that the Masai may have basic genetic traits different from Caucasians with regard to serum proteins and lipoproteins.

Comment - Over-all steady, although somewhat slow, progress was made in the field of atherosclerosis during 1969. The future looks brighter than in previous years as prior research and study is beginning to yield dividends. Still

needed is a suitable laboratory model resembling the disease state and there is room for considerable development in the understanding of atheromata pathogenesis. The most encouraging development is the significant gains that have been made toward therapeutic alleviation of the necrological end result of atherosclerosis.

References

1. L. H. Krut, Brit. J. Clin. Pract. 22, 517 (1968).
2. D. F. Davies, J. Atheroscler. Res., 10, 253 (1969).
3. K. J. Kingsbury, Nature, 224, 146 (1969).
4. F. G. Schettler and G. S. Boyd (eds.), "Atherosclerosis," Elsevier, New York, 1969.
5. C. W. M. Adams, 2nd International Symposium on Atherosclerosis, Chicago, 1969, Abstr. p. 3.
6. Y. H. Abdulla, C. W. M. Adams and O. B. Bayliss, J. Atheroscler. Res., 10, 229 (1969).
7. E. B. Smith, 2nd International Symposium on Atherosclerosis, Chicago, 1969, Abstr. p. 4.
8. P. T. Kuo, Metabolism, 18, 631 (1969).
9. P. T. Kuo, Med. Counterpoint, 1, 47 (1969).
10. R. S. Clements, Jr., A. D. Morrison and A. I. Winegrad, Science, 166, 1007 (1969).
11. R. S. Levy and C. E. Day, 2nd International Symposium on Atherosclerosis, Chicago, 1969, Abstr. p. 14.
12. Y. Stein and O. Stein, ibid, Abstr. p. 12.
13. R. S. Lees, Circulation, 39-40 (Suppl. 3), 15 (1969).
14. J. Slack and G. L. Mills, 2nd International Symposium on Atherosclerosis, Chicago, 1969, Abstr. p. 15.
15. J. Dyerberg, Metabolism, 18, 50 (1969).
16. L. A. Lewis, J. D. Battle, Jr. and I. H. Page, Circulation, 39-40 (Suppl. 3), 16 (1969).
17. A. Studer, 2nd International Symposium on Atherosclerosis, Chicago, 1969, Abstr. p. 2.
18. J. F. Mustard, ibid, Abstr. p. 5.
19. J. E. French, ibid, Abstr. p. 6.
20. A. B. Chandler, ibid, Abstr. p. 6.
21. L. Jorgensen, ibid, Abstr. p. 7.
22. K. Kjeldsen, P. Astrup and J. Wanstrup, J. Atheroscler. Res., 10, 173 (1969).
23. P. Astrup, K. Kjeldsen and J. Wanstrup, 2nd International Symposium on Atherosclerosis, Chicago, 1969, Abstr. p. 7.
24. K. Kjeldsen, ibid, Abstr. p. 30.
25. C. Garbarsch, M. E. Matthiessen, P. Helin and I. Lorenzen, J. Atheroscler. Res., 9, 283 (1969).
26. P. Helin, I. Lorenzen, C. Garbarsch and M. E. Matthiessen, ibid, 9, 295 (1969).
27. W. H. Strain, C. G. Rob, W. J. Pories, R. C. Childers, M. F. Thompson, Jr., J. A. Hennessen and F. M. Graber,

Nat. Bur. Stand. (U.S.) Spec. Publ., 312(1), 128 (1969).
28. G. Rose, 2nd International Symposium on Atherosclerosis, Chicago, 1969, Abstr. p. 24.
29. R. S. Filo, C. H. Sloan, L. Weatherbee and W. J. Fry, ibid, Abstr. p. 36.
30. H. A. Schroeder, J. Nutr., 97, 237 (1969).
31. J. Stamler, D. M. Berkson, H. A. Lindberg, W. A. Miller, E. L. Stevens, R. Soyugenc, T. J. Tokich and R. Stamler, 2nd International Symposium on Atherosclerosis, Chicago, 1969, Abstr. p. 30.
32. M. R. Malinow and A. Perley, J. Atheroscler. Res., 10, 107 (1969).
33. P. J. Nestel, H. M. Whyte and D. S. Goodman, J. Clin. Invest., 48, 982 (1969).
34. B. A. Kottke, Circulation, 40, 13 (1969).
35. W. Raab, Med. Counterpoint, 1, 29 (1969).
36. M. Friedman, S. O. Byers and S. R. Elek, Proc. Soc. Exp. Biol. Med., 131, 759 (1969).
37. S. M. Grundy and E. H. Ahrens, Jr., J. Lipid Res., 10, 91 (1969).
38. S. M. Grundy, E. H. Ahrens, Jr. and J. Davignon, ibid, 10, 304 (1969).
39. R. I. Levy, D. S. Fredrickson, P. O. Kwiterovich, W. V. Brown, H. R. Sloan, C. J. Glueck, A. M. Gotto, J. LaRosa and T. Langer, Clin. Res., 17, 474 (1969).
40. G. Christakis and S. H. Rinzler, in "Atherosclerosis," F. G. Schettler and G. S. Boyd (eds.), Elsevier, New York, 1969.
41. W. E. Connor, D. T. Witiak, D. B. Stone and M. L. Armstrong, J. Clin. Invest., 48, 1363 (1969).
42. N. Spritz and M. A. Mishkel, ibid, 48, 78 (1969).
43. E. M. Jepson, M. F. I. Fahmy, P. E. Torrens, J. D. Billimoria and N. F. Maclagan, Lancet, 2, 1315 (1969).
44. P. J. Scott and P. J. Hurley, J. Atheroscler. Res., 9, 25 (1969).
45. D. B. Hunninghake, D. R. Tucker and D. L. Azarnoff, Circulation, 39, 675 (1969).
46. D. B. Hunninghake, Mod. Med., 37, 100 (1969).
47. M. Albert and M. J. Stansell, Metabolism, 18, 635 (1969).
48. F. Schaffner, Gastroenterology, 57, 253 (1969).
49. M. G. Kokatnur, G. T. Malcom and R. D. Martinez, Metabolism, 18, 73 (1969).
50. D. Zakim and R. H. Herman, J. Atheroscler. Res., 10, 91 (1969).
51. R. J. Cenedella, J. J. Jarrell and L. H. Saxe, ibid, 8, 903 (1968).
52. W. R. Ruegamer, N. T. Ryan, D. A. Richert and W. W. Westerfeld, Biochem. Pharmacol., 17, 613 (1969).
53. C. G. McKerron, R. L. Scott, S. P. Asper and R. I. Levy, J. Clin. Endocrinol. Metab., 29, 957 (1969).

54. H. S. Sodhi, L. Horlick and B. Kudchodkar, Circulation, 39-40 (Suppl. 3), 24 (1969).
55. S. Grundy, New York Academy of Sciences Meeting, 1969.
56. M. M. Best and C. H. Duncan, J. Atheroscler. Res., 10, 103 (1969).
57. G. Hartmann and G. Forster, ibid, 10, 235 (1969).
58. D. Berkowitz, Circulation, 39-40 (Suppl. 3), 44 (1969).
59. M. Mancini, P. Oriente, M. Motolese and R. Cerqua, Circulation 39-40 (Suppl. 3), 139 (1969).
60. P. Oriente, M. Motolese, R. Cerqua and M. Mancini, Int. Z. Klin. Pharmakol. Ther. Toxikol., 2, 348 (1969).
61. A. R. Timms, L. A. Kelly, R. S. Ho and J. H. Trapold, Biochem. Pharmacol., 18, 1861 (1969).
62. L. D. Bechtol and W. L. Warner, Angiology, 20, 565 (1969).
63. B. M. Cohen, J. Clin. Pharmacol., 9, 45 (1969).
64. N. Meric and K. Ozkan, Ankara Univ. Tip Fak. Mecm., 21, 1063 (1968).
65. R. J. Jones and L. Dobrilovic, Circulation, 39-40 (Suppl. 3), 12 (1969).
66. T. M. Parkinson, K. Gundersen and N. A. Nelson, ibid, 39-40 (Suppl. 3), 19 (1969).
67. A. Rubulis, M. Rubert and W. W. Faloon, Fed. Proc., Fed. Amer. Soc. Exp. Biol., 28, 268 (1969).
68. M. Ito, W. E. Connor, E. J. Blanchette, C. R. Treadwell and G. V. Vahouny, J. Lipid Res., 10, 694 (1969).
69. F. M. Berger, J. F. Douglas, B. J. Ludwig and S. Margolin, J. Pharmacol. Exp. Ther., 170, 371 (1969).
70. F. M. Berger, J. F. Douglas, G. G. Lu and B. J. Ludwig, Proc. Soc. Exp. Biol. Med., 132, 293 (1969).
71. D. Kritchevsky, P. Sallata and S. A. Tepper, ibid, 132, 303 (1969).
72. J. W. Drake, R. H. Bradford, M. McDearmon and R. H. Furman, Metabolism, 18, 916 (1969).
73. J. W. Barnhart, J. A. Sefranka and D. D. McIntosh, Fed. Proc., Fed. Amer. Soc. Exp. Biol., 28, 268 (1969).
74. Med. News, 348, 1 (1969).
75. C.-C. Wu, T.-S. Huang and C.-J. Hsu, Amer. Heart J., 77, 657 (1969).
76. J. W. Barnhart, J. A. Sefranka and D. E. Bublitz, Proc. Soc. Exp. Biol. Med., 130, 1161 (1969).
77. H. Fukushima and H. Nakatani, J. Atheroscler. Res., 9, 65 (1969).
78. H. Fukushima and H. Nakatani, ibid, 9, 57 (1969).
79. K. Toki and H. Nakatani, Advan. Exp. Med. Biol., 4, 491 (1969).
80. G. Porcellati, D. Giorgini and E. Toja, Lipids, 4, 190 (1969).
81. M. L. Black, G. Rodney and D. B. Capps, Biochem. Pharmacol., 17, 1803 (1968).
82. O. Linet, Arzneim.-Forsch., 19, 1899 (1969).
83. L. J. Lerner, D. N. Harris, E. Yiacas, R. Hilf and

I. Michel, Fed. Proc., 28, 268 (1969).
84. D. Harman, J. Atheroscler. Res., 10, 77 (1969).
85. R. A. Salvador, C. Atkins, S. Haber and C. Kozma, Fed. Proc., Fed. Amer. Soc. Exp. Biol., 28, 677 (1969).
86. T. H. Chung, G. V. Vahouny and C. R. Treadwell, J. Atheroscler. Res., 10, 217 (1969).
87. G. Bazzano, Proc. Soc. Exp Biol. Med., 131, 1463 (1969).
88. G. Tamasi, J. Borsy and A. Patthy, Biochem. Pharmacol., 17, 1789 (1968).
89. I. Chibata, K. Okumura, S. Takeyama and K. Kotera, Experientia, 25, 1237 (1969).
90. T. Kamiya, Y. Saito, M. Hashimoto and H. Seki, Tetrahedron Lett., 53, 4729 (1969).
91. J. Ditzel and J. Dyerberg, J. Atheroscler. Res., 10, 5 (1969).
92. L. M. Morrison, J. J. Quilligan, Jr., K. Murata, O. A. Schjeide and L. Freeman, Int. Congr. Gerontol, Proc., 7th 2, 73 (1966).
93. T. A. Anderson and R. D. Bowman, Proc. Soc. Exp. Biol. Med., 130, 665 (1969).
94. R. L. Buchanan, V. Sprancmanis and R. A. Partyka, J. Med. Chem., 12, 1001 (1969).
95. B. B. Chaudhari, J. Pharm. Sci., 58, 366 (1969).
96. H. Buchwald, J. Atheroscler. Res., 10, 1 (1969).
97. T. L. Kopjas, U.S. Patent 3,463,854 (1969).
98. P. M. Shah, R. Gramiak, A. G. Adelman and E. D. Wigle, Circulation 39-40 (Suppl. 3), 183 (1969).
99. B. Sigel, R. J. Gibson, K. V. Amatneek, W. R. Felix, Jr. and G. L. Popky, ibid, 39-40 (Suppl. 3), 187 (1969).
100. International Conference on Bioelectric Impedance held by New York Academy of Sciences, Sept. 1969.
101. O. Turpeinen, 2nd International Symposium on Atherosclerosis, Chicago, 1969, Abstr. p. 43.
102. J. Stamler, ibid, Abstr. p. 44.
103. H. W. Schnaper, H. K. Schoch and K. Detre, Circulation, 39-40 (Suppl. 3), 180 (1969).
104. M. F. Oliver, 2nd International Symposium on Atherosclerosis, Chicago, 1969, Abstr. p. 44.
105. J. P. Strong and H. C. McGill, Jr., J. Atheroscler. Res., 9, 251 (1969).
106. J. K. Lloyd and O. H. Wolff, ibid, 10, 135 (1969).
107. W. B. Kannel, W. P. Castelli and P. M. McNamara, Minn. Med., 52, 1225 (1969).
108. W. S. Collens and G. B. Dobkin, Circulation, 39-40 (Suppl. 3), 5 (1969).
109. W. S. Collens, Med. Counterpoint, 1, 53 (1969).
110. L. A. Lewis, K.-J. Ho, C. B. Taylor, B. Mikkelson and K. Biss, Fed. Proc., 28, 553 (1969).

Chapter 18. Steroids and Biologically Related Compounds

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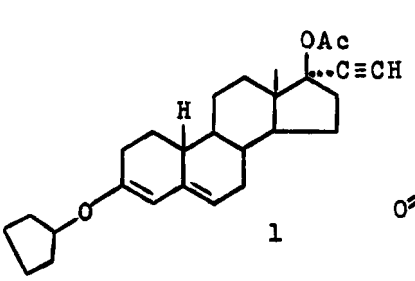
I. REPRODUCTION

A. Female Contraceptives - During the latter part of this year, congressional investigators publicized the potential dangers in using oral contraceptives. This, however, did not shed any light on providing a safe and reliable method to control the burgeoning world population. Experts in this field realize that the ideal contraceptive has not been found but feel the benefits from using available drugs far outweigh the risks. To the individual, oral contraceptives provide a reliable and easy means to family planning, while to society restricting population growth leads to less strain on natural resources and less pollution of the environment.

The search goes on for new and equally effective contraceptive which have fewer side effects than those presently being used.

Human Testing - Among estrogen-progestin combinations, a combination of quinestrol (17α-ethynyl estradiol 3-cyclopentyl ether) and quingestanol (1) was clinically effective given orally, once-a-month.[1] Quinestrol is stored in the body fat and released slowly throughout the cycle causing infertility.[2] A once-a-month oral contraceptive may be well suited for underdeveloped countries where medical supervision is limited. Norgestrel (17α-ethynyl-18-methyl-19-nortestosterone), in combination with ethynyl estradiol showed good contraceptive efficacy given 21 days in a cycle.[3] The lower daily doses of progestin (0.5 mg) and of estrogen (0.05 mg) may decrease untoward reactions



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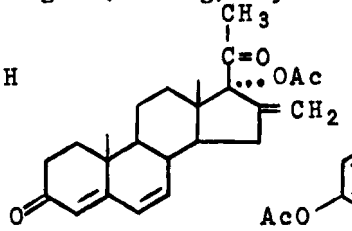

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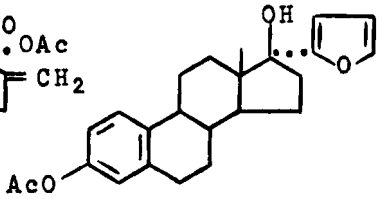

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without affecting efficacy. Compound 2 (7 mg/day) and mestranol (0.075mg/day), was used in a sequential tablet with good results.[4] Trials with new regimens of existing drugs[5,6] and methods for choosing oral contraceptives[7] have also been published.

Further attempts at developing continuous, low-dose, progestational contraceptives were reported. Medroxyprogesterone acetate[8] and norethisterone enanthate[9] were found to be released slowly enough and in sufficient concentration to be effective when injected every three months. Other means for delivering constant low doses of progestin are being developed. Megestrol acetate[10,11] was found to provide suitable contraceptive action when implanted subcutaneously in silastic capsules. Daily, continuous oral administration of 0.5 mg of either megestrol acetate[12] or chlormadinone acetate (CAP)[13,14] prevented pregnancy. Norgestrel, 0.05 mg and norethisterone acetate, 0.3 mg were also effective when administered daily.

The mechanism of action of contraceptive drugs was the subject of an

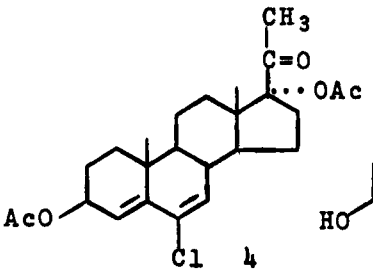
excellent review.[15] It is clear that in man ovulation inhibition by estrogen-progestin combinations is not necessary for contraception.[16] Inhibiting implantation through a drug-induced altered endometrium is another possible means of preventing pregnancy. In fact, if contraceptives do inhibit luteal function[17,18,19] then the latter mechanism may be more important than ovulation inhibition since the indirect criteria for determining ovulation are luteal dependent. It also appears urinary excretion of LH and pregnanediol may not be reliable indicators of ovulation.[20]

There is good evidence that continuous low dose progestin therapy does not prevent pregnancy by blocking ovulation[13,14,16] although norethisterone enanthate is claimed to do so.[9] Changes in spinnbarkeit of the cervical mucus which prevents sperm penetration have also been claimed to play a role in preventing pregnancy.[12] Megestrol acetate, at an ineffective contraceptive dose of 0.25 mg/day, however, still caused cervical mucus changes which have been considered hostile to sperm.[14] The endometrial effects of low dose progestins, like the progestin-estrogen combinations, therefore, could account for the infertile state.[12]

Biological side effects of progestins and estrogens, not directly related to contraception, continued to be reported. Thrombophlebitis has occurred less frequently with combined preparations containing 0.05 mg of estrogen than with those containing 0.075 or 0.1 mg.[21] High doses of estrogen used for suppressing lactation were also implicated in thromboembolic episodes.[22] Subjects with a history of toxemia or pre-existing renal disease were susceptible to the hypertensive effects of the combination contraceptives.[23] Women using norethynodrel-mestranol combination had abnormal levels of glucose after 24 months of therapy but returned to normal after an additional 12 months of use.[24] Diabetic patients with endometrial carcinoma receiving medroxyprogesterone acetate required higher doses of insulin.[25] The diabetogenic effect of contraceptives was not demonstrated in an insulin B-chain induced hyperglycemic rat.[26] Increased iron, and iron binding capacity was found with combined contraceptive therapy especially if the progestin was a 17α-acetoxy-20-ketosteroid.[27,28] Oral contraceptives also increased serum levels of copper and ceruloplasmin.[29] On the other hand, evidence was presented showing long term use of norethynodrel and mestranol caused no frank toxicity,[30] while in a short-term study, patients on placebo had similar "side-effects" to those found in women on contraceptive therapy.[31] Quinestrol, in a toxicological study in dogs, produced no pathological changes.[32]

In women, single doses of mestranol or quinestrol were excreted primarily as ethynyl estradiol.[33] The rate of excretion was constant for mestranol. It was biphasic for quinestrol; fast initially followed by a slower rate.

Animal Testing - In rats, the contraceptive effects of AY-11483 (3) were potentiated by three progestins, AY-11440 (4), CAP, and medrogestone (6,17α-dimethyl-6-dehydroprogesterone).[34] Use of 3 alone or in combination with 4 was more effective in preventing ovulation and implantation than was ethynyl estradiol alone or in combination with CAP or medrogestone. Only if therapy was started early in the cycle was ovulation inhibited; if started later, implantation was prevented. The pyrazole 5 given to rats orally after ovulation also prevented implantation.[35] Deladroxone (6) caused



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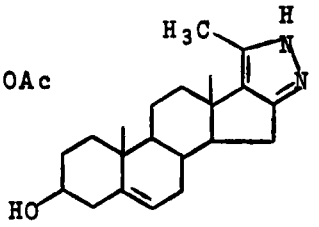

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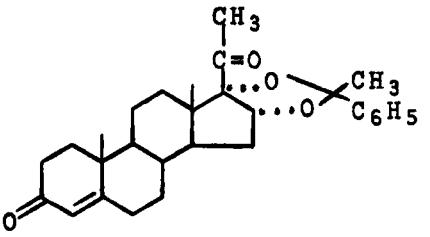

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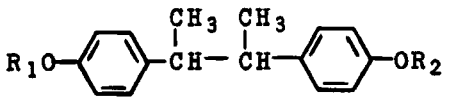
sterility in female mice.[36] It is not clear, however, whether loss of libido or inhibition of ovulation is the main contraceptive effect. Dehydroepiandrosterone (DHA) was shown to interrupt pregnancy in rats by causing expulsion of the eggs from the uterus.[37] Evidence was presented to show that the ovary converts DHA to an estrogen.

A number of mono and dibasic ether derivatives of trans-2-methylstilbene, trans-α,α'-dimethylstilbene, α-methylbibenzyl and α,α'-dimethylbibenzyl were tested in mice for estrogenic, anti-estrogenic (intravaginal or s.c. injection) and post-ovulatory contraceptive (s.c. injection) activities.[38] Most compounds had anti-estrogenic but no anti-fertility activity when applied intravaginally. Compounds 7 a-f had both estrogenic and anti-fertility activities. Only 7a had a greater anti-fertility effect than estrogenic potency. 1-Nitro-2 (4-ethylphenyl)-1,2-diphenylethylene demonstrated 100% contraceptive effect at doses with low estrogenic action.[39]



7a R_1=H, R_2=$(CH_2)_2NEt_2$

b R_1=H, R_2=$(CH_2)_2NMe_2$

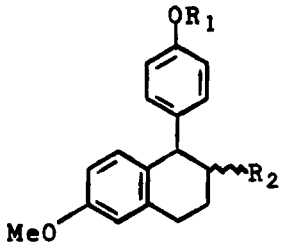
c R_1=H, R_2=$(CH_2)_2$-N⟨ ⟩

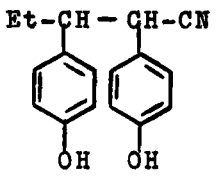
d R_1=H, R_2=$(CH_2)_2$-N⟨ ⟩

e R_1=CH_3, R_2=$(CH_2)_2NMe_2$

f R_1=R_2=$(CH_2)_2NEt_2 \cdot HCl$

Compound 8a was an effective contraceptive in rats at doses as low as 0.01 mg/day.[40] The free phenol 8b augmented the uterotrophic response of estradiol, while its ethers, although estrogenic at contraceptive doses, antagonized the uterotrophic response of estradiol.





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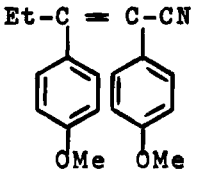


10

(SC-3296)

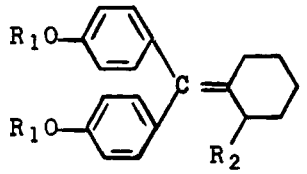
8a R_1=$(CH_2)_2$N⟨ ⟩, R_2=C_6H_5; cis

b R_1=H, R_2=C_5H_9; trans

The above results indicate that estrogenic, anti-estrogenic and contraceptive activities can be produced by the same molecule, but a 1:1:1 relationship in each activity is not apparent.

The mode of contraceptive action of these drugs can be due to actions on the ovary, fallopian tubes, uterus or blastocysts. Erythro-α-ethyl-α'-methyl-4,4'-dihydroxybibenzyl is an active antifertility agent when administered to mice prior to implantation.[41] In mice, this compound has high estrogenic activity if administered in divided doses, but low activity if injected as a single dose. The drug causes the ova to be locked in the fallopian tubes, indicating that its tubal contraceptive action is more important than its uterine effect.[42] In rats, 9 and 10 are estrogenic but accelerate the rate of tubal transport of ova.[43] Prevention of uterine maturation, however, has not been ruled out as the prime contraceptive effect.

Implantation could be inhibited by preventing progesterone priming of of the uterus by affecting luteal steroidogenesis. 5-Bromo-2-thienyl ethyl ketone thiosemicarbazone decreased ovarian progesterone concentration and thereby inhibited decidualization.[44] Compounds 11a-d were found to concentrate in luteal tissue in mice.[45] These compounds were also shown to prevent pregnancy and to prevent the histochemical demonstration of Δ^5-3-hydroxysteroid dehydrogenase, one of the required enzymes for converting pregnenolone to progesterone. In rats, however, 11a did not seriously inhibit adrenal steroidogenesis.[46] The mode of action of these agents appeared most likely related to their estrogenic activity.[46,47] Both ergocornine and D-6-methyl-8-cyanomethylergoline were effective in preventing implantation.[48,49] Ergocornine was claimed to block the hypophysial support of luteal function.[48]



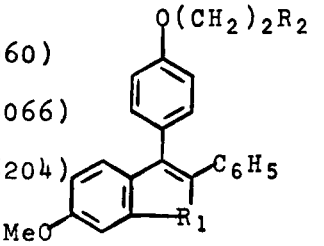
11

a R_1=H, R_2=H (F6060)

b R_1=Ac, R_2=H (F6066)

c R_1=H, R_2=CH_3 (F6204)

d R_1=Ac, R_2=CH_3 (F6103)



12a R_1=$(CH_2)_2$, R_2= N⟨ ⟩

b R_1=CH_2, R_2= NEt_2

U-11100A (12a), was found in vitro to prevent normal embryo development.[50] This is not considered the prime contraceptive effect because in vivo antizygotic concentrations of drug are not attained in the fallopian tube. This agent has the two additional contraceptive actions of increasing tubal transport of ova and decreasing uterine development. A related compound U-11555A (12b), appears to block implantation by inhibiting an estrogen-dependent function in the blastocyst.[51] ORF-4563 (2-methyl-3-ethyl-4-phenyl-Δ^4-cyclohexenecarboxylic acid sodium salt) is a potent antizygotic drug in rabbits.[52] The rate at which the drug is metabolized is an important factor in determining the dose required to prevent pregnancy.

Since in mice and rats estrogens are required for implantation and can also be contraceptives, it is difficult to pinpoint by what contraceptive mechanism weak estrogens work, i.e., by blocking the estrogen trigger for implantation or by acting as estrogens. Estrogens cause uterine growth through a two step mechanism.[53] The hormone associates spontaneously with

an extranuclear protein forming an 8s complex and is then transported to the nucleus. Diethylstilbestrol, ethynyl estradiol and estrone acetate showed greater affinity in vitro for isolated rabbit uterine receptor than estradiol.[54] Alteration in the D-ring by 16-hydroxylation enhances binding while substitution at the C_2 position inhibits binding. Somewhat different affinities were found with mouse uterine receptors where estrone and estriol had one tenth the affinity of estradiol.[55]

Estradiol can also effect uterine growth through a proposed labilization of the lysosomal membrane and subsequent release of biogenic amines increasing cyclic 3',5' AMP(CAMP).[56,57] CAMP, in addition to affecting uterine development, also prevents pregnancy in mice.[58]

The relationships among the 8s receptor, release of biogenic amines, and estrogen-triggered implantation in rodents must still be clarified. This information could help determine whether the drugs described above prevent pregnancy by a truly anti-estrogenic action.[59,60]

B. Ovulation Induction - Anti-estrogenic activity, in addition to causing infertility, has also been claimed to be a means for inducing ovulation.[61] It is postulated that the anti-estrogenic property of clomiphene blocks the feedback action of estrogen on the hypothalamic-pituitary axis, thus bringing about a release of gonadotropin which leads to ovulation. Clomiphene reverses the watery characteristic of the cervical mucus which is an anti-estrogenic action in man.[62] The estrogenic characteristics of clomiphene, however, cannot be ruled out as the ovulatory stimulus because F6066 (11b), which like clomiphene is anti-estrogenic in rats,[47] induces ovulation in women without any change in the cervical mucus.[63] Induction of ovulation in anovulatory, pseudopregnant rats by clomiphene, F6066, and stilbestrol, is additional evidence that estrogenic activity may account for this response.[47] Further evaluation of data demonstrating the anti-estrogenic activity of clomiphene on the hypothalamic-pituitary axis must await clarification of the results showing these centers do not preferentially concentrate estrogen as does the uterus.[64]

C. Male Contraceptives - In rats, cyproterone acetate (13) 10 mg/day for 48 days had no effect on sexual behavior and ejaculations, but mated females did not become pregnant.[65] Creation of hostile environment for the storage of sperm or alteration in the seminal fluid due to the anti-androgenic action of 13 are possible causes of sterility. Another anti-androgen, SKF 7690 (43a), caused sterility in mice after 35 days of treatment with a daily dose of 12.5 mg.[66] Spermatogenesis was not effected but decreased libido and reduction in reproductive capacity have not been ruled out. 3-Chloro-1,2-propanediol (U-5897) caused reversible sterility in rats, guinea pigs and monkeys.[67] U-5897 may produce a local ischemia resulting in epithelial desquamation blocking the caput epididymis. In cases where the germinal epithelium does degenerate, the cause is pressure from fluid accumulation in the testis. In rats there is some evidence that U-5897 caused a metabolic disturbance within the spermatozoa.[68]

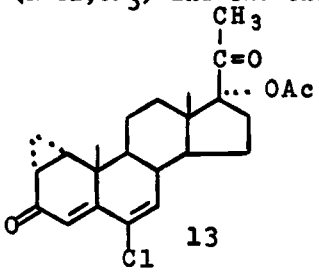
D. Estrus Synchronization - Megestrol acetate (12 mg/kg/day for 7 days) synchronized ovulation in 50% of the treated rats.[69] Both medroxy-progesterone acetate and chlorpromazine were also able to delay ovulation in rats, presumably by a similar mechanism to that of megestrol acetate.[70]

CAP, melengestrol acetate, and megestrol acetate were shown to synchronize heat in cattle. There was, however, a high incidence of anovulatory heats indicating that the first post-treatment period would be associated with low fertility.[71]

Estradiol, in ewes, initially induced ovulation and then maintained the corpus luteum but inhibited subsequent ovulations with continued treatment.[72] In heifers, estradiol caused luteal regression.[73]

II. PROGESTATIONAL AGENTS

The 4-chlorosteroid 14 and several related compounds showed high oral progestational activity.[74] The 4-amino-derivatives 15 (R=1-pyrrolidino, etc.) were also reported to be orally active.[75] The 16-methylene compounds 16 (R=Cl,CH_3) and the chemical precursor $\Delta^{1,4,6}$-trienes were highly active



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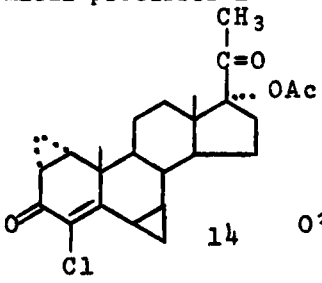

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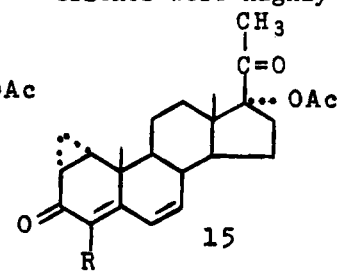

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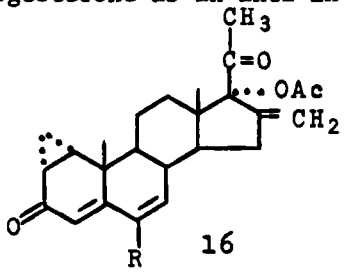
in the Clauberg test (i.m.).[76] The 13-ethyl-9β,10α-steroids 17[77], 18[78] and the 3-deoxysteroid 19[79] had considerable oral progestational activity. The 10-azasteroid 20 was less active than norethisterone but equal to progesterone as an anti-LH compound.[80]



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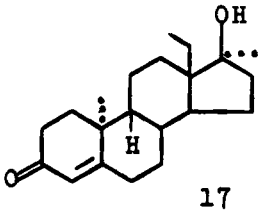

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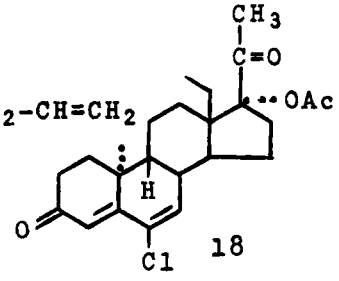

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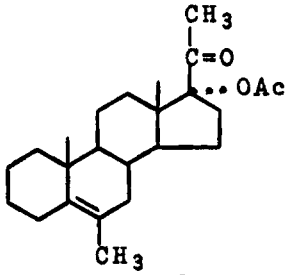
The potent progestin, Sch 12600 (21) or progesterone maintained pregnancy in rats castrated after implantation. Estrone permitted pregnancy maintenance with doses of 21 (0.25 mg/kg) or progesterone (2 mg/kg) which by themselves were ineffective.[81]



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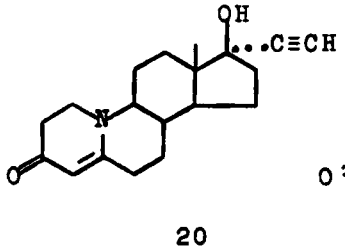

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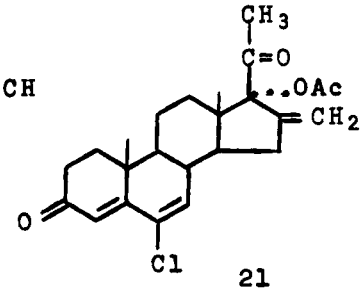


21

In rats, progesterone at an optimal ratio to estrogen can facilitate the release of LH and induce ovulation.[82] A new retroprogesterone derivative, RO-4-8345, was also found to trigger ovulation in several cases of Stein-Leventhal Syndrome.[83]

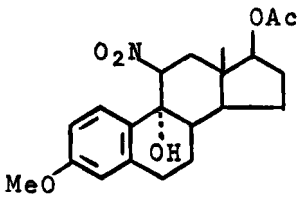
Norethisterone, 17α-hydroxyprogesterone, medroxyprogesterone acetate and ethynodiol diacetate caused tumor regression in 13 of 27 patients with advanced carcinoma of the endometrium.[84,85] A six year study established that Enovid did not initiate or accelerate precancerous growth and may have had an opposite effect.[86]

Dimethisterone, megestrol acetate and progesterone prevented the appearance of experimental pulmonary emphysema.[87] The mechanism for the antiemphysematous action is not known but the bronchomotor effect was ruled out as a principal action.

Progesterone was reported to be an immunosuppressant causing otherwise resistent monkeys to become susceptible to viral tumors.[88] Pregnancy or treatment with progesterone (2 mg) and estrone (0.5μg) maintained skin implanted in a rat uterus.[89] Evidence was presented which suggests that the maintenance of the implant is not an immunosuppressant action of progesterone and estrone. Estradiol benzoate was shown to be the critical factor in the maintenance of a skin autograft to the rat uterus.[90] Estradiol pretreatment was shown to protect mice against total body irradiation.[91,92] Estrone and estriol were less effective, while estradiol valerate was ineffective.

III. ESTROGENS

The synthesis of a group of 9α-hydroxy-11β-nitro estrogens was reported.[93] In castrated rats, 22 had 3 times the potency of ethynyl estradiol in producing vaginal cornification. The 17α-isoxazolyl steroid 23 had about one-third the estrogenic activity of mestranol in the Allen-Doisy assay (p.o. or s.c.).[94] The estrogenic potencies of a series of 3-alkoxyestra-1, 3,5(10)-trien-17β-ols were found to be less than that of estradiol because



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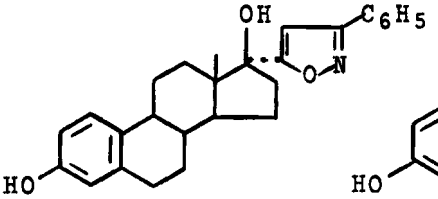

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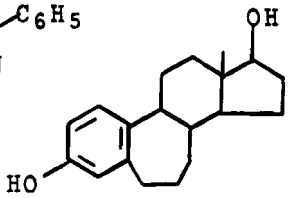


24

of decreased affinity for the receptor.[95] Compared to the parent ether, introduction of a hydroxyl group at the end of the alkyl chain increases potency, indicating that this hydroxyl group is important for binding at receptor site. The synthesis of (±) B-homoestradiol (24) was reported and it had about 2-3% the estrogenic activity of estradiol in vivo.[96] A series of 3-deoxy-16-haloestrones 25 (R=Cl,Br,I) exhibited increased lipodiatic/estrogenic ratios compared to that of 3-deoxyestrone.[97]

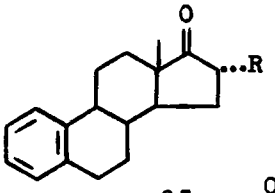
F6066 (11b), was found ineffective in the treatment of prostatic cancer.[98] Diethylstilbestrol, however, was beneficial for the treatment of the subjective symptoms and therefore the drug of choice in advanced stages of tumor metastases.

Stilbestrol and hexestrol caused atrophy of external endometriosis.[99] Estradiol, estrone, and progestagens used in birth control pills were thought to be contraindicated because they may stimulate growth of uterine leiomyomas in cases of endometriosis.

Estrogen was considered useful for replacement therapy in post-menopausal women.[100,101] It was also shown that with increasing age the livers of post-menopausal women continue to metabolize estrogen in a normal way.[102] Stilbestrol at relatively high doses (5 mg daily) caused no vascular accidents or phlebitis in more than 100,000 patients.[99]

IV. CORTICOIDS

A detailed study of the structure-activity relationship among important [17α,16α-d]-oxazolino analogs of corticoids has been published.[103] The



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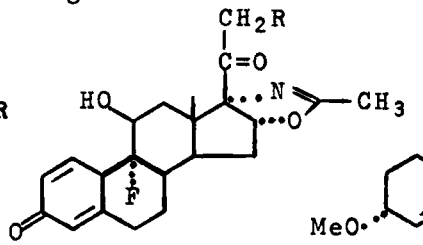
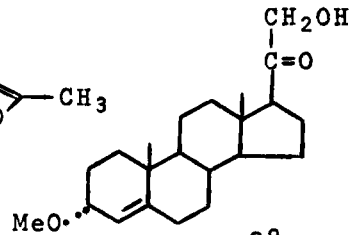


26 R=OAc
27 R=H



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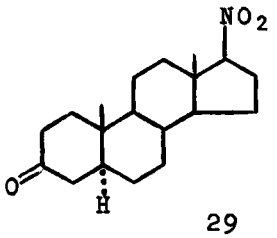
2'-methyloxazoline ring in a large number of steroids such as 26 potentiates anti-inflammatory (cotton pellet assay) and gluconeogenic activity and reduces mineralocorticoid activity. Compound 27 still retains about half of the anti-inflammatory activity of 26 while being almost devoid of mineralocorticoid activity. In contrast, the 3α-methoxy compound 28 was an active mineralocorticoid without any glucocorticoid activity.[104] A series of nitrate esters of steroid hormones were prepared, and the 17-nitrate of dihydrotestosterone (29) had anti-inflammatory activity in the range of prednisolone as measured by the granuloma-inhibition assay.[105] Tomatine, a steroidal alkaloid glycoside, exhibited low anti-inflammatory activity in carrageenan-induced rat paw edema while its aglycone, tomatidine, was ineffective.[106] The anti-inflammatory, anti-diuretic, and gastric ulcer healing properties of carbenoxolone sodium (30), a triterpene derivative from licorice root, has been published.[107]

Enhancement of the anti-inflammatory effect of corticoids by estrogens was reported.[108,109] Addition of estrogens produced a 3-20 fold reduction of the previously established requirement of corticosteroids for the successful control of skin diseases in women.[108]



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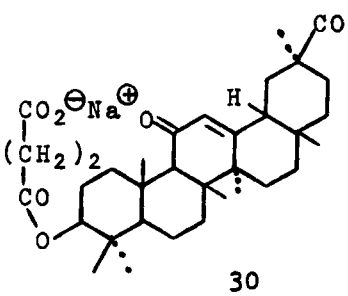

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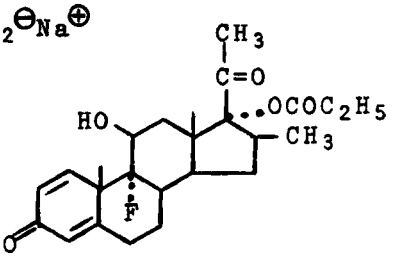


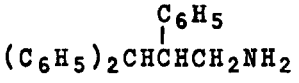
31

The comparative topical anti-inflammatory potency of 31 to betamethasone 17-valerate was directly related to the formulation used.[110] The depot-effects of paramethasone acetate (20 mg for 2 weeks, 40 mg for 3 weeks)[111] and the successful use of preseasonal Depo-Medrol for the treatment of grass pollen hay fever was described.[112] It was observed that CAP (30 mg/day, 10-11 days) causes a slight reduction in the adrenal function in man. This can be explained as an expression of a corticoid-like effect of the steroid on the adrenotropic center.[113] Similar effects with other potent progestins were noticed earlier. The effect of adrenal steroid therapy in neurological diseases,[114] and the problems related to growth were reviewed.[115] Comparison of corticoids in the corticoid glucose tolerance test showed betamethasone, paramethasone and fluprednisolone to have a weak effect on carbohydrate metabolism. The authors suggest that this finding offers particular possibilities for corticoid therapy in diabetics.[116] It would be interesting to learn if this concept will be validated in further clinical studies.

Several compounds related to 32 were described as specific inhibitors of aldosterone biosynthesis in vitro without altering deoxycorticosterone and corticosterone levels.[117]

V. ANDROGENS

The 2-thia-A-norsteroid 33 was found to have androgenic potency in the order of testosterone.[118] This interesting finding suggests (a) that



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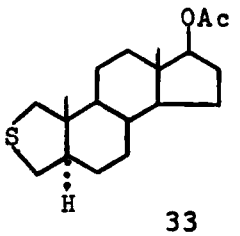

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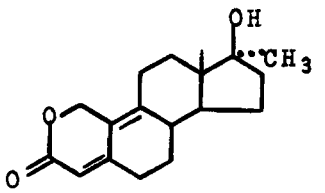

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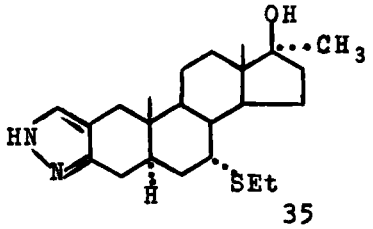
steric effects and not electronic factors are important in connection with C_2 and/or C_3 in androgens, and (b) it is possible to prepare biologically active nor-steroids by substituting -S- for -CH=CH-. The 2-oxasteroid 34 was reported to possess very high activity relative to methyltestosterone (androgenic: 22-47 x; anabolic: 550 x).[119] The structure-activity relationship among a large number of 7α-alkylthioandrostanes showed the pyrazole 35 to have the best separation of anabolic from androgenic activity (ratios: 5.6 p.o., 4.8 s.c.).[120] The biological profile of 2α,3α-epithio-5α-androstane-17β-ol and related compounds was reviewed.[121,122] The AB-dinorsteroid 36 was devoid of androgenic or anti-androgenic activity.[123] The 13-ethyl-9,10-retrosteroid 37 had the oral anabolic activity of methandienone.[77]



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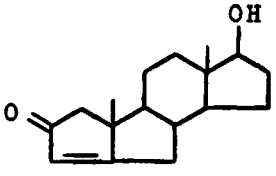

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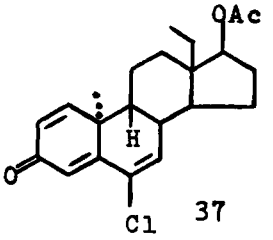
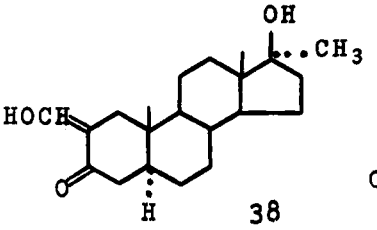


37

The solubilities of some steroids in water and simulated intestinal fluid are related to increased anabolic and androgenic efficiency.[124]

Additional evidence was obtained that dihydrotestosterone, and not testosterone, is the "active androgen" in the prostate where it is found in highest concentration.[125,126]



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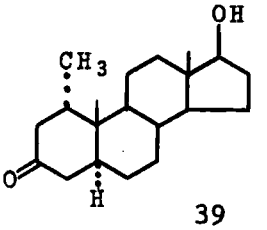

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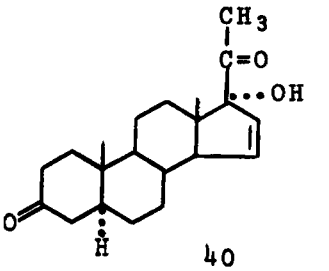

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The use of oxymetholone (38)[127,128], methenolone (39)[127], dromostanolone (2α-methyl-dihydrotestosterone)[127], and methyltestosterone[129] caused remission of aplastic anemia which in some cases was permanent (1-5 years). The use of 38 and 39 caused less virilization than the other androgens used.

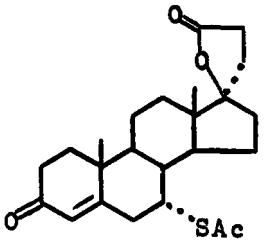
VI. ANTI-ANDROGENS

The search to find androgen antagonists without the feminizing side effects for the treatment of benign prostatic hypertrophy (BPH), androgen-dependent acne and prostatic cancer has stimulated research in this area in the last few years. Cyproterone acetate (13) remains the most thoroughly studied compound in this field. Compound 13 at 50 mg/day for up to 15 months was effective or partially effective in relieving the symptoms of BPH in 11 of 13 patients.[130] The anti-androgenic activity of 13 can be attributed to a decreased accumulation of testosterone in the rat ventral prostate,[131] possibly as a result of its binding to the "androgen receptor".[132,133] Compound 13 also exerts a strong anti-androgenic action on the seminal vesicles but does not effect the androgen dependent mating behavior in gonadectomized, sexually-experienced male rats.[134] Compound 13 was shown to inhibit androgen-induced erythropoiesis in mice.[135]

Other steroids effective in the treatment of advanced BPH were 17α-hydroxyprogesterone caproate (6 of 9 patients, daily dose not reported) and chlormadinone acetate (2 of 3 patients).[136]

The 15-dehydrosteroid 40[137] and spironolactone (41)[138] were reported to antagonize the androgen-induced weight increase in the accessory sex organs of castrate rats. The non-steroid 42 exhibited interesting anti-androgenic activity in rats, but the effective dose was very close to the toxic dose.[139] Pretreatment of immature rats with phenobarbital or chlordane increased liver metabolism of exogenous androgen causing a decreased size of the seminal vesicles.[140]

The use of 43a (50-600 mg/day, p.o.) was temporarily effective in the treatment of acne and idiopathic hirsutism.[141] The related 43b was effective in reducing sebum production in the rat, but it also exhibited weak, but definite uterotrophic activity.[142,143] The stimulating effect of testosterone propionate (0.5 mg/day) on the rate of sebum secretion on immature castrated rats was blocked by 13 (5 mg/day).[144] In man,



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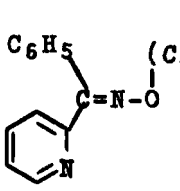

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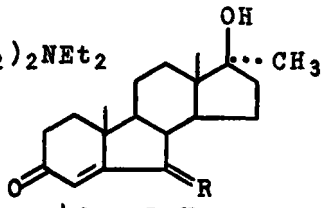


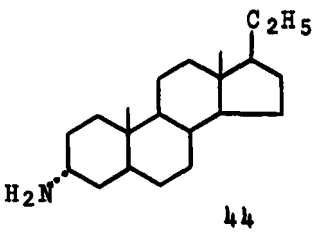
43a R=H_2

b R=spirocyclopropyl

application of 13 (100-200mg/day, p.o., or 1% in oil-water emulsion, topically) was effective for the treatment of acne.[145] At these high doses, however, gynecomastia and menstrual disturbances were observed.

VII. STEROIDS ACTIVE ON THE CENTRAL NERVOUS SYSTEM

The aminopregnane 44, related to funtumine, showed tranquilizing and sedative properties in mice without cardiac or respiratory depression.[146] Pancuronium bromide (45) was clinically useful as an i.v. anaesthetic, even



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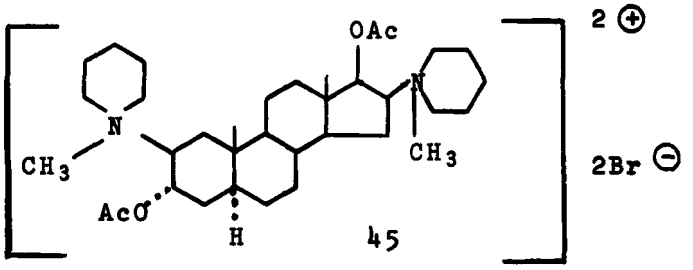
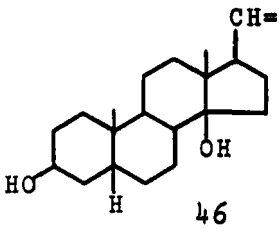


45

with patients suffering with liver diseases or renal insufficiency.[147] The neuromuscular blocking activities of some bis-quaternary salts of N,N'-dimethylconessine were described.[148]

VIII. CARDENOLIDES, BUFADIENOLIDES

Basic esters derived from cardenolides (cf. 46) possess inotropic activities in the anaesthetized dog, but are less toxic than the corresponding cardenolides.[149] Spironolactone (41) was found to protect rats against otherwise fatal digitoxin poisoning.[150]



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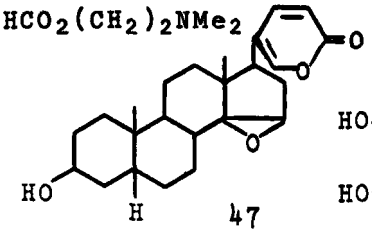

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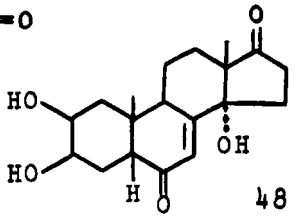

48

Resibufagenine (47), with no 14β-hydroxy group, showed cardiotonic effects in hypotensive dogs.[151] The tumor inhibitory activities of the dihydrobufadienolides withaferin A, withacnistin,[152] and the bufadienolide, hellebrigenin-3,5-diacetate[153] were reported.

IX. STEROIDS WITH ANTIBACTERIAL ACTIVITY

A number of aminosteroids related to 17β-amino-3,5-androstadiene were active against Trichophyton asteroids and Penicillium citrinum QM-1226.[154] Several 4-aza-20α-aminopregnanes showed activity against gram-positive bacteria in vitro.[155] A series of des-A-aminoandrostane derivatives showed activity against mycobacteria in vitro.[156] The steroidal antibiotic, fusidic acid and its sodium salt were both capable of penetrating intact human skin at a rate similar to the glucocorticoids.[157] Holotoxin, an antifungal steroid glycoside, isolated from sea cucumber showed high activity against pathogenic fungi in vitro.[158]

X. INSECT HORMONES

The rapid development in the field of insect hormones, has been reviewed.[159,160] Ponasteride A (Ponasterone A 3β-glucopyranoside)[161] and Viticosterone E (identical with pterosterone)[162] were among the more interesting phytoecdysones isolated during the year. The stimulating effect of insect metamorphosing steroids on protein synthesis in the mouse liver was demonstrated.[163,164] It appears that the hydroxylated cholestane or stigmastane side chain is not essential for this type of activity, since rubrosterone (48) is also active. A structure-activity correlation of various steroids with antisclerotization effect on Pyrrhocoris apterus L. larvae was reported.[165] The synthesis of antheridiol, a steroidal fungal sex hormone was described.[166]

XI. REVIEWS

Extensive reviews on control of human fertility appeared in the British Medical Bulletin[167] and in a WHO technical report.[168] Current research on steroid contraception was summarized.[169,170,171] A review on induction of ovulation in anovulatory women was published.[172] An extensive review on androgen-anabolic agents[173] and androgen metabolism[174] appeared in press. The chemistry and biology of steroidal antibiotics[175] and the microbiological degradation of steroids[176] was reviewed.

The IUPAC-IUB revised tentative rules for nomenclature of steroids were published.[177,178]

REFERENCES

1. M. Masquero-Topete, E. Berman, J. Soberson, and J. J. Calderon, Fert. Steril., 20, 884 (1969).
2. T. Mischler, D. Gawlak, T. Giannina, and A. Meli, Proc. Soc. Exptl. Biol. Med., 132, 323 (1969).
3. G. L. Foss, J. Reprod. Fert., 18, 59 (1969).
4. R. Sterba, Prakt. Lekar, 49, 15 (1969); Int. Pharm. Abstracts, 6, 3229 (1969).
5. W. G. McBride, Arzneim. Forsch., 19, 1271 (1969).
6. L. E. Moses, J. W. Goldzieher, and I. G. Moses, Fert. Steril, 20, 715 (1969).
7. R. P. Dickey and C. H. Dorr, Obstet. Gynec., 33, 273 (1969).
8. R. A. Lee, Am. J. Obstet. Gynec., 104, 130 (1969).
9. J. Zanartu and C. Navarro, Obstet. Gynec., 31, 627 (1968).
10. H. Croxatto, S. Diaz, R. Vera, M. Etchart, and P. Atria, Am. J. Obstet. Gynec., 105, 1135 (1969).
11. H. J. Tatum, E. M. Coutinho, J. A. Filho, and A. R. S. Sant' Anna, ibid., 105, 1139 (1969).
12. H. A. van Leusden, J. Reprod. Fert., 19, 537 (1969).
13. A. Gutierrez-Najar, H. Marquez-Monter, V. Cortes-Gallegos, J. Giner-Velazquez, and J. Martinez-Manautou, Am. J. Obstet. Gynec., 102, 1018 (1968).
14. E. Mears, M. P. Vessey, L. Andolsek, and A. Oven, Brit. Med. J., 2, 730 (1969).
15. E. Diczfalusy, Am. J. Obstet. Gynec., 100, 136 (1968).
16. A. D. Papanicolaou, J. A. Loraine, and M. C. N. Jackson, Acta endocr. (Kbh), 62, 477 (1969).
17. V. H. Erb and K. S. Ludwig, Gynaecologia, 155, 309 (1965).
18. E. Østergaard and J. Starup, Acta endocr. (Kbh), 57, 386 (1968).
19. A. S. Watnick, S. Tolksdorf, J. Kosieroswki, and I. I. A. Tabachnick, Excerpta Med. (Amst.) Internat. Congr. Series, 132, 889 (1967).
20. E. Diczfalusy, U. Goebelsmann, E. Johannisson, K. G. Tillinger, and L. Wide, Acta endocr. (Kbh), 62, 679 (1969).
21. E. C. G. Grant, Brit. Med. J., 4, 73 (1969).
22. D. G. Daniel, Am. Heart J., 78, 720 (1969).
23. M. H. Weinberger, R. D. Collins, A. J. Dowdy, G. W. Nokes, and J. A. Luetscher, Ann. Internal Med., 71, 891 (1969).
24. W. N. Spellacy, R. P. Bendel, W. C. Buki, and S. A. Birk, Fert. Steril., 20, 892 (1969).
25. H. Gershberg, E. Zorrilla, A. Hernandez, and M. Hulse, Obstet. Gynec., 33, 383 (1969).
26. R. L. Fenichel, R. M. Purse, H. E. Alburn, and R. A. Edgren, Acta endocr. (Kbh), 62, 438 (1969).
27. M. Mardell, C. Symmons, and J. F. Zilva, J. Clin. Endocr. Metab., 29, 1489 (1969).
28. M. Thein, G. H. Beaton, H. Milne, and M. J. Veen, Canad. Med. Ass. J., 101, 73 (1969).
29. M. Briggs, J. Austin, and M. Staniford, Nature, 225, 81 (1970).
30. E. T. Tyler, S. L. Cole, M. Levin, and J. Elliot, Fert. Steril., 20 871 (1969).

31. R. Aznar-Ramos, J. Giner-Velazquez, R. Lara-Ricalde, and J. Martinez-Manautou, Am. J. Obstet. Gynec., 105, 1144 (1969).
32. E. Schwartz, J. A. Tornaben, and G. C. Boxill, Toxicology and Applied Pharmacology, 14, 487 (1969).
33. K. I. H. Williams, Steroids, 13, 539 (1969).
34. U. K. Banik, C. Revesz, and F. Herr, J. Reprod. Fert., 18, 509 (1969).
35. S. K. Saksena and R. R. Chaudhury, ibid., 19, 177 (1969).
36. S. R. Munshi and S. S. Rao, Ind. J. Med. Res., 57, 1475 (1969).
37. M. J. K. Harper, Endocrinology, 84, 229 (1969).
38. C. W. Emmens, D. J. Collins, J. J. Hobbs, B. G. Miller, and W. H. Owen, J. Pharmacol. Exper. Therap., 165, 52 (1969).
39. P. Queval, B. Falconnet, J. G. Giraud, A. Krikorian-Manoukian, D. Courmarcel, J. C. Bondiou, and N. P. Buu-Hoi, Chim. Therap., 4, 1 (1969).
40. D. Lednicer, D. E. Emmert, S. C. Lyster, and G. W. Duncan, J. Med. Chem., 12, 881 (1969).
41. B. G. Miller, and C. W. Emmens, J. Endocr., 45, 9 (1969).
42. K. W. Humphrey and C. W. Emmens, J. Reprod. Fert., 20, 247 (1969).
43. E. F. Nutting and F. J. Saunders, Proc. Soc. Exptl. Biol. Med., 131, 1326 (1969).
44. N. R. Mason, F. C. Tinsley, and R. L. Cochrane, Endocrinology, 85, 831 (1969).
45. L. E. Appelgren, J. Reprod. Fert., 19, 185 (1969).
46. T. Giannina, B. Steinetz, M. Butler, J. Chart, and R. Carney, Biology of Reproduction, 1, 397 (1969).
47. A. S. Watnick, and R. O. Neri, Acta endocr. (Kbh), 59, 611 (1968).
48. E. Turolla, G. Baldratti, E. Scrascia, and G. Ricevuti, Experientia, 25, 415 (1969).
49. K. Rezabek, M. Semonsky, and N. Kucharczyk, Nature, 221, 666, (1969).
50. J. T. TenBroeck and R. L. Brinster, J. Reprod. Fert., 18, 201 (1969).
51. K. Yoshida and J. M. Craig, Fert. Steril., 20, 674 (1969).
52. A. S. Yard, L. P. Juhasz, and R. M. Grimes, J. Pharmacol. Exper. Therap. 167, 105 (1969).
53. E. V. Jensen, T. Suzuki, M. Numata, S. Smith, and E. R. Sombre, Steroids, 13, 417 (1969).
54. S. G. Korenman, ibid., 13, 163 (1969).
55. L. Terenius, ibid., 13, 311 (1969).
56. C. M. Szego and J. S. Davis, Life Sciences, 8, 1109 (1969).
57. C. M. Szego and J. S. Davis, Mol. Pharmacol., 5, 470 (1969).
58. W. L. Ryan and D. M. Coronel, Am. J. Obstet. and Gynec., 105, 121 (1969).
59. C. W. Emmens and B. G. Miller, Steroids, 13, 725 (1969).
60. L. Martin, ibid., 13, 1 (1969).
61. S. Roy, R. B. Greenblatt, and V. B. Mahesh, Acta endocr. (Kbh), 47, 657 (1964).
62. V. Ruiz-Velasco, R. B. Uriza, B. I. Conde, and E. Salas, Fert. Steril., 20, 829 (1969).
63. T. Sato, Y. Ibuki, M. Hirono, M. Igarashi, and S. Matsumoto, ibid., 20, 965 (1969).
64. R. E. Whalen and R. A. Maurer, Proc. Natl. Acad. Sci., 63, 681 (1969).
65. R. E. Whalen and W. G. Luttge, Nature, 223, 633 (1969).

66. H. L. Saunders, J. Tomaszewski, J. Pauls, and W. Zuccarello, Endocrinology, 85, 960 (1969).
67. R. J. Ericsson and N. D. Connor, Abstracts Society for the Study of Reproduction Second Annual Meeting p. 25 (1969).
68. E. Smojlik and M. C. Chang, ibid, p. 25 (1969).
69. J. P. Bennett, D. K. Vallance, and B. H. Vickery, J. Reprod. Fert., 16, 159 (1968).
70. U. K. Banik and F. Herr, Canad. J. Physiol. Pharmacol., 47, 573 (1969).
71. D. H. Hale and R. B. Symington, J. Reprod. Fert., 18, 193 (1969).
72. E. L. Piper and W. C. Foote, ibid., 16, 253 (1968).
73. M. A. Brummer, L. E. Donaldson, and W. Hansel, J. Dairy Sci., 52, 1849 (1969).
74. R. Wiechert, H. Laurent, and H. Hofmeister, Rev. Soc. Quim. Mex., XIII, 73A (1969).
75. H. Laurent, G. Schulz, and R. Wiechert, Chem. Ber., 102, 2570 (1969).
76. E. L. Shapiro, T.L. Popper, L. Weber, R. Neri, and H. L. Herzog, J. Med. Chem., 12, 631 (1969).
77. S. J. Halkes and R. van Moorselaar, Rec. Trav. Chim. Pays-Bas, 88, 752 (1969).
78. R. van Moorselaar and S. J. Halkes, ibid., 88 737 (1969).
79. A. P. Shroff, J. Med. Chem., 12, 548 (1969).
80. D. Bertin and J. Peronnet, Bull. Soc. Chim. Fr., 117 (1969).
81. E. Melczer, R. O. Neri, and A. S. Watnick, Fed. Proc., 28, 772, (1969).
82. S. Y. Ying and R. K. Meyer, J. Reprod. Fert., 20, 279 (1969).
83. I. Gerhard and O. Stamm, Gynaecologia, 168, 25 (1969).
84. B. Kneale and J. Evans, Med. J. Austral, 2, 1101 (1969).
85. J. G. Peck and D. A. Boyes, Am. J. Obstet. Gynec., 103, 90 (1969).
86. J. E. Ayre, F. C. Reyner, W. B. Fagundes, and J. M. LeGuerrier, Obstet. Gynec., 34, 545 (1969).
87. D. M. Aviado and G. R. McKinney, Pharmacol. Res. Comm., 1, 283 (1969).
88. J. S. Munroe, J. Nyitray, D. Queen, S. Toppel, and W. F. Windle, J. Immunol., 28, 768 (1968).
89. R. Russo, J. M. Yun and A. S. Watnick, Fed. Proc., 28, 581 (1969).
90. R. Prager and J. Zipper, Am. J. Obstet. Gynec., 103, 86 (1969).
91. J. S. Thompson, E. L. Simmons, M. K. Crawford, and C. D. Severson, Radiation Res., 40, 70 (1969).
92. J. S. Thompson, C. D. Severson, and R. W. Reilly, Radiation Res., 40, 46 (1969).
93. G. Baldratti, W. Barbieri, A. Consonni, R. Sciaky, E. Scrascia, and G. K. Suchowsky, Experientia, 25, 1018 (1969).
94. H. Laurent and G. Schulz, Chem. Ber., 102, 3324 (1969).
95. C. Liarakos and M. May, Endocrinology, 84, 1247 (1969).
96. E. Galantay and H. P. Weber, Experientia, 25, 571 (1969).
97. W. F. Johns, J. Med. Chem., 12, 885 (1969).
98. H. E. Carstensen, C. E. Olsen, V. Sele, I. Brorson, E. Jensen, N. I. Nissen, and J. Fabricius, Nord. Med., 81, 111 (1969).
99. K. J. Karnaky, Obstet. Gynec., 33, 597 (1969).
100. S. Gould, Clin. Med., 76, 18 (1969).
101. H. S. Acken, Obstet. Gynec., 34, 46 (1969).
102. B. J. Procope and H. Adlercreutz, Acta endocr. (Kbh), 62, 461 (1969).

103. G. Nathansohn, C. R. Pasqualucci, P. Radaelli, P. Schiatti, D. Selva, and G. Winters, Steroids, 13, 365 (1969).
104. K. Irmscher, G. Cimbollek, H. Halpaap, and H. G. Kraft, Rev. Soc. Quim. Mex., XIII, 72A (1969).
105. F. Hodosan, O. Mantsch, I. Jude, D. Breazu, B. Cuparencu, and N. Sterescu, Arzneim. Forsch., 19, 684 (1969).
106. R. B. Fildermann and B. A. Kovacs, Brit. J. Pharmac., 37, 748 (1969).
107. "A Symposium on Carbenoxolone Sodium," J. M. Robson and F. M. Sullivan, Ed., Butterworths, London, 1968.
108. A. S. Spangler, H. N. Antoniades, S. L. Sotman, and R.M. Inderbitizin, J. Clin. Endocr. Metab., 29, 650 (1969).
109. J. Guidollet and P. Louisot, Acta endocr. (Kbh), 62, 468 (1969).
110. M. J. Busse, P. Hunt, K. A. Lees, P.N.D. Maggs, and T. M. McCarthy, Brit. J. Derm., 81, Suppl. 4, 103 (1969).
111. J. G. Rausch-Stroomann and R. Petry, Arzneim. Forsch., 19, 767 (1969).
112. M. A. Ganderton, J. Brostoff, and A. W. Frankland, Brit. Med. J., 1, 357 (1969).
113. P. M. Reisert and A. v.z. Muhlen, Arzneim Forsch., 19, 1279 (1969).
114. A. A. Eisen and J. W. Norris, Canad. Med. Assoc. J., 100, 27, 66 (1969).
115. Brit. Med. J., 1, 393 (1969).
116. G. Fiegel and D. Kukwa, Int. Z. fur Klin. Pharm., 2, 94 (1969).
117. B. Blank, W. A. Zuccarello, S. R. Cohen, G. J. Grishmuth, and D. Scaricaciottoli, J. Med. Chem., 12, 873 (1969).
118. M. E. Wolff and G. Zanati, ibid., 12, 629 (1969).
119. E. F. Nutting and D. W. Calhoun, Endrocrinology, 84, 441 (1969).
120. H. Kaneko, K. Nakamura, Y. Yamato, and M. Kurokawa, Chem. Pharm. Bull., 17, 11 (1969).
121. T. Miyake and A. Tanaka, Ann. Rept. Shionogi Res. Lab., 19, 21 (1969).
122. T. Miyake, T. Komeno, and K. Takeda, ibid, 19, 39 (1969).
123. W. G. Dauben, D. J. Ellis and W. H. Templeton, J. Org. Chem., 34, 2297 (1969).
124. R. Huttenrauch and I. Keiner, Arch. Pharm., 301, 856 (1968).
125. N. Bashirelaki, G. J. Chader, and C. A. Villee, Biochem. Biophys. Res. Commun., 37, 976 (1969).
126. A. Kowarski, J. Shalf, and C. J. Migeon, J. Biol. Chem., 244, 5269 (1969).
127. L. Sanchez-Medal, A. Gomez-Leal, L. Duarte, and M. Guadelupe Rico, Blood, 34, 283 (1969).
128. S. J. Silink and B. G. Firkin, Australasian Ann. Med., 17, 224 (1968); Int. Pharm. Abstracts, 6, 3073 (1969).
129. D. Naveh and T. Jankielowitz, Harefuah, 76, 511 (1969); Int. Pharm. Abstracts, 6, 3061 (1969).
130. W. W. Scott and J. C. Wade, J. Urology, 101, 81 (1969).
131. J. Geller, O. van Damme, G. Garabieta, A. Loh, J. Rettura, and E. Seifter, Endocrinology, 84, 1330 (1969).
132. J. M. Stern and A. J. Eisenfeld, Science, 166, 233 (1969).
133. S. Fang, K. M. Anderson, and S. Liao, J. Biol. Chem., 244, 6584 (1969).
134. R. E. Whalen and D. A. Edwards, Endrocrinology, 84, 155 (1969).
135. E. A. Mirand, J. H. Groenewald, G. M. Kenny, and G. P. Murphy, Experientia, 25, 1104 (1969).

136. J. Geller, A. Angrist, K. Nakao and H. Newman, J. Am. Med. Ass., 210, 1421 (1969).
137. T. L. Popper, J. N. Gardner, R. Neri, and H.L. Herzog, J. Med. Chem., 12, 393 (1969).
138. S. L. Steelman, J. R. Brooks, E. R. Morgan, and D. J. Patanelli, Steroids, 14, 449 (1969).
139. F. J. Villani, R. F. Tavares, and C. A. Ellis, J. Pharm. Sci., 58, 138 (1969).
140. W. Levin, R. M. Welch, and A. H. Conney, Steroids, 13, 155 (1969).
141. S. Dalla Pria, R. B. Greenblatt, and V. B. Mahesh, J. Invest. Dermat., 52, 348 (1969).
142. H. Saunders and F. J. Ebling, J. Invest. Dermat., 52, 163 (1969).
143. F. J. Ebling, Proc. roy. Soc. Med., 62, 890 (1969).
144. A. Archibald and S. Schuster, ibid., 62, 887 (1969).
145. K. Winkler, Arch. klin. exp. Derm., 233, 296 (1968).
146. O. Foussard-Blanpin, Ann. Pharm. Fr., 27, 191 (1969).
147. E. Stojanov, Arzneim Forsch, 19, 1723 (1969).
148. D. Busfield, K. J. Child, A. J. Clarke, B. Davis, and M. G. Dodds, Brit. J. Pharm. Chemother., 32, 609 (1968).
149. J. Bowler and R. Clarkson, Rev. Soc. Quim. Mex. XIII., 68A (1969).
150. H. Selye, J. Jelinek and M. Krajny, J. Pharm. Sci., 58, 1055 (1969).
151. J. M. Leigh and A. D. S. Caldwell, J. Pharm. Pharmac., 21, 708 (1969).
152. S. M. Kupchan, W. K. Anderson, P. Pollinger, R. W. Doskotch, J. A. Saenz Renauld, H. K. Schnoes, A. L. Burlingame, and D. H. Smith, J. Org. Chem., 34, 3858 (1969).
153. S. M. Kupchan, R. J. Hemingway, and J. C. Hemingway, ibid., 34, 3894 (1969).
154. Y. Nagai, Chem. Pharm. Bull., 17, 1749 (1969).
155. F. H. Jawad, Ph.D. Thesis, University of Mississippi, 1969; Dissert. Abstr. 1059-B (1969).
156. C. Rufer, Ann., 726, 145 (1969).
157. C. F. H. Vickers, Brit. J. Derm., 81, 902 (1969).
158. S. Shimada, Science, 163, 1462 (1969).
159. C. E. Berkoff, Quart. Rev., 23, 372 (1969).
160. K. Nakanishi, Bull. Soc. Chim. Fr., 3475 (1969).
161. H. Hikino, S. Arihara, and T. Takemoto, Tetrahedron, 25, 3909 (1969).
162. H. Rimpler, Tetrahedron Lett., 329 (1969).
163. T. Otaka, M. Uchiyama, T. Takemoto, and H. Hikino, Chem. Pharm. Bull., 17, 1352 (1969).
164. T. Otaka, M. Uchiyama, S. Okui, T. Takemoto, H. Hikino, S. Ogawa, and N. Nishimoto, ibid., 17, 2426 (1969).
165. H. Velgova, V. Cerny, F. Sorm, and K. Slama, Coll. Czech. Chem. Commun., 34, 3354 (1969).
166. J. A. Edwards, J. S. Mills, J. Sundeen, and J. H. Fried, J. Amer. Chem. Soc., 91, 1248 (1969).
167. British Medical Bulletin, 26, No. 1., (1970).
168. Report No. 424, World Health Organization Technical Report Series, Geneva, Switzerland, (1969).
169. E. T. Tyler, J. Reprod. Fert., 20, 319 (1969).
170. C. Dodds, Clin. Pharm. Therap., 10, 147 (1969).

171. B. Tuttle, Can. Pharmac. J., 102, 102 (1969).
172. "Induction of Ovulation", By R. P. Shearman, C. C. Thomas, Publisher, Springfield, Ill., 1969.
173. "Androgens and Anabolic Agents", By J. A. Vida, Academic Press, Inc., New York, N.Y., 1969.
174. J. S. Strauss and P. E. Pochi, Arch. Derm., 100, 621 (1969).
175. Antibiotiki, 554 (1969) (in Russian).
176. C. Horhold, K. h. Bohme, and K. Schubert, Z. fur. Allg. Mikrobiol., 9, 235 (1969).
177. Steroids, 13, 278 (1969).
178. J. Org. Chem., 34, 1517 (1969).

Chapter 19. Peptide Hormones

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This survey will attempt to cover the significant advances in the two years since this subject was last reviewed in this series. Progress in this time has been rapid, and many exciting new developments have appeared. Several general reviews have become available.[1-4]

HORMONES OF THE PITUITARY AND HYPOTHALAMUS

The hypothalamus - Abundant evidence has been accumulated that the secretion of the neurohypophyseal hormones is under the control of substances produced in the hypothalamus. These substances, releasing or inhibiting factors, travel to the anterior pituitary by means of the pituitary portal circulation, and there cause (or inhibit) the degranulation of specific cell types which contain the tropic hormones. Several general reviews have appeared.[5-8] The most exciting development in this area is the establishment of PCA-His-Pro-NH_2 as the structure of porcine thyrotropin releasing factor (TRF).[9-11] One molecule of this peptide will liberate 20,000 molecules of TSH from pituitary slices. Porcine growth hormone releasing factor (GRF) has been purified 56,000 times; this material appears to be a peptide containing 15 amino acid residues - Lys, His, Ser, Glu(5), Ala(4), Val and Leu(2).[12] It is similar to TRF in that it contains no free amino or carboxyl groups. This material causes specific degranulation of the somatotrophic cells of the anterior pituitary, as demonstrated by electron microscopy.[13] There is an indication that the hypothalamic releasing factors may exert their effect on the pituitary through the mediation of cyclic AMP.[14]

The anterior pituitary - Rapid progress has been made in the chemistry of growth hormone (GH). The proceedings of two conferences on GH have appeared.[15,16] The amino acid sequence of human GH (HGH) has been published,[17] as well as partial sequences for porcine,[18] equine,[19] and bovine[20] GH. The amino acid sequences at the carboxyl ends of these molecules are very similar:

Human:	-Arg-Ile-Val-Gln-Cys-Arg——Ser-Val-Glu-Gly-Ser-Cys-Gly-Phe
Bovine:	-Arg-Val-Met-Lys-Cys-Arg-Arg-Phe-Gly-Glu-Ala-Ser-Cys-Ala-Phe
Porcine and Equine:	-Arg-Val-Met-Lys-Cys-Arg-Arg-Phe-Val-Glu-Ser-Ser-Cys-Ala-Phe

The questions of structure-activity and species specificity have received much attention. Bovine GH (BGH) exists as two different species,[21] neither of which is active in the human. These two chains differ by an amino-terminal tetrapeptide. Limited digestion of BGH with trypsin, chymotrypsin, or papain[22] produces fragments with HGH activity. One of these active fragments appears to have a molecular weight of about 5000. Interesting chemical modifications of HGH by Li's group[23] have shown that the two disulfide bridges are not necessary for GH (rat tibia) or prolactin (PL) (pigeon cropsac) activity, while an intact tryptophan is essential for PL but not for GH activity. The results of a large study on treatment of hypopituitary dwarfs

with HGH have been summarized,[24] and indicate that initially promising results frequently diminish with continued treatment. Antibody formation may be a cause of this effect. Human placental lactogen, which has recently been renamed "human chorionic somato-mammotropin (HCS),[25] has been shown to have anabolic effects when administered to hypopituitary dwarfs in large doses.[15] Research on this substance was recently reviewed.[26] The C-terminal amino acid sequence is identical to that shown above for HGH except that Met replaces Ile.

Radioimmunoassay techniques[27-29] for GH have greatly increased the sensitivity and speed of GH measurement; three of these methods have been compared.[30] Investigators using these methods must always bear in mind that specificity for binding of proteins to antibodies is based on parts of hormone molecules that may have no relationship to the presence or absence of an intact biological activity site. This has been demonstrated strikingly for the calcitonins (see below). Used with proper caution, however, these methods enable elegant studies to be made, such as that in which the locus in the rat hypothalamus (the ventromedial nucleus) responsible for GH release was located.[31] Cyclic AMP has been implicated as a mediator in the stimulation of cell division by GH.[32]

The exact nature of prolactin (PL) in the human remains an open question. In lower species, PL and GH are clearly different molecules, although their biological and immunological properties show considerable overlap. The two hormones from the rat can be separated readily by gel electrophoresis.[33] The complete amino acid sequence of ovine PL was announced by Li's laboratory.[34] It contains 198 amino acids (vs. 188 for HGH), and sequences near the carboxyl end are very similar. Rat PL has been highly purified, and appears to contain 193 amino acids.[35] It does not cross-react immunologically with the bovine, ovine, and rabbit hormones. Radioimmunoassay techniques for PL have made possible studies of serum PL levels during different reproductive states[36] and of the effects of hypothalamic estrogen implantation.[37]

Recent research on gonadotropins is summarized in the proceedings of a conference[38] and in a review.[39] Complete structures of follicle stimulating hormone (FSH) and lutenizing hormone (LH) are not known although work continues on ovine[40] and bovine[41] LH. The current status of radioimmunoassay techniques for these hormones has been reviewed.[42,43]

Thyroid-stimulating hormone (TSH), a glycoprotein of molecular weight about 25,000 has been purified from bovine, porcine, and human pituitaries.[44] The human and bovine hormones have a common C-terminal pentapeptide sequence, His-Tyr-Lys-Ser-Tyr, while the porcine hormone lacks the C-terminal tyrosine. The human hormone may be two different molecules having a common C-terminal portion. Initial structural work on bovine TSH shows that it may be quite similar to LH.[45] A radioimmunoassay for human TSH has been developed,[46] and the involvement of cyclic AMP in the function of TSH has been implied.[47]

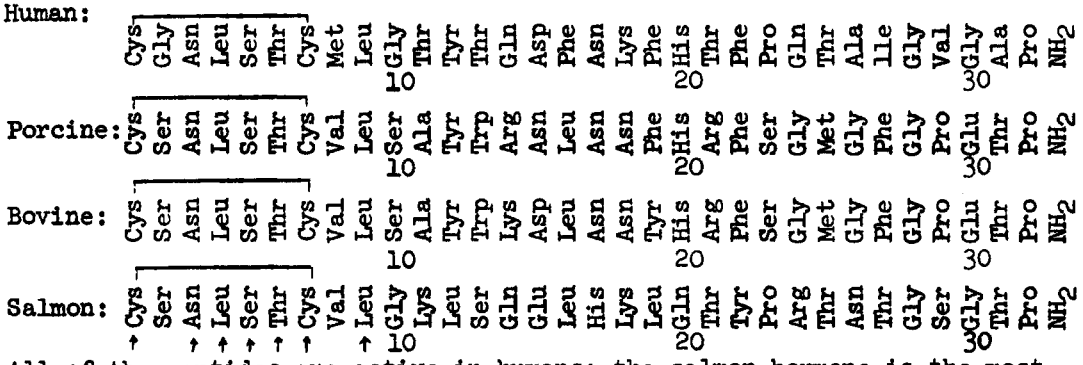
New syntheses of peptides related to adrenocorticotropic hormone (ACTH) continue to appear. Among the most recent are a synthesis of ACTH 1-23 amide[48] and of several ornithine analogs.[49] Clinical studies on several of the short chain peptides have been published,[50-52] as well as new procedures for preparation of ACTH antisera.[53,54] A need for calcium has been shown in the ACTH stimulation of adrenal cortical adenyl cyclase.[55] Syntheses of α-MSH[56] and monkey MSH[57] have appeared.

The posterior pituitary - A massive review of the neurohypophyseal hormones

has appeared.[58] This field continues to be active, with new syntheses of analogs appearing readily. The laboratories of du Vigneaud[59] and Rudinger[60] remain very active. Solid phase peptide systhesis has been used very successfully for a large number of syntheses in this area.[59,61-64] The unusual acetone derivatives of oxytocin and vasopressin have been synthesized.[65] The work of Sawyer and Manning on evolutionary aspects of the posterior pituitary hormones is particularly interesting.[62,66] Snake[67] and salmon[68] posterior pituitary peptides have been characterized. NMR conformational studies,[69] new active analogs without disulfide bridges,[70,71] and 4-substituted analogs with high oxytocic but no antidiuretic activity[72] have been reported. A carbamyl derivative,[73] an analog with an enlarged ring,[74] and Arg[8]-vasopressinoic acid[75] have been found to inhibit the normal peptides. This last compound inhibits the vasopressin-stimulated adenyl cyclase of kidney medulla, but not that of kidney cortex, which is stimulated by parathormone. Progress in vasopressin radioimmunoassay has been reported[76,77] Oxytocin and vasopressin are stable in the pulmonary circulation,[78] in contrast to some other peptide hormones.[79] The biosynthesis and release of vasopressin and neurophysin have been reviewed.[80]

HORMONES OF CALCIUM METABOLISM

Calcitonin - Remarkable progress has been made in the last two years in the chemistry and biology of the hypocalcemic hormone calcitonin (CT, thyrocalcitonin). Several reviews have appeared.[81-84,133] The amino acid sequence of porcine CT was announced almost simultaneously by several groups, and those of human, bovine, and salmon CT followed soon thereafter. The human,[85] porcine,[86] and salmon[87] hormones have been synthesized. All the hormones are 32-residue peptides having an amino-terminal 7-residue cystine ring and a C-terminal proline amide. Only nine residues are identical in all species. Six of these are in the ring, and the others are Leu[9], Gly[28] and Pro[32]. The structures are as follows:[88,89] (residues marked in the salmon sequence are invariant in all species).



All of the peptides are active in humans; the salmon hormone is the most active of all (35 x human CT activity in the rat) and has a prolonged action. An interesting point concerns Met[8] in the human hormone. If it is oxidized to the sulfoxide, all activity is lost. Yet it can be replaced by valine, as in the other species, with retention of full activity. Human CT has also been isolated as a biologically active dimer, which consists of antiparallel chains cross-linked by two disulfide bridges.[90]

CT is secreted by the C-cells of the thyroid[91] and causes net movement of calcium into bone by inhibiting bone resorption and inhibiting calcium excretion by the kidney.[92] Renal clearance of CT has been studied.[93] This hormone will probably play an important role in the treatment of osteoporosis and Paget's disease.[94] Radioimmunoassays for human[95] and porcine[96] CT have been developed, and studies on the antigenic site[97] and a comparison of bioassay and immunoassay[98] have appeared. The structural features required for biological activity seem to be located at the ends of the chain, while the antigenic site in the center, in the variable part of the chain. A consequence of this is the observation that while the different species of CT are biologically active in humans, they do not cross-react with human CT in immunoassays. The bovine and porcine hormones are very similar throughout, and they do cross-react mutually.[98] Furthermore, while oxidation of Met^8 in the human CT to the sulfoxide destroys biological activity, it does not impair antibody binding. Extreme caution must thus be used in interpreting the results of immunoassays for CT, expecially if mixed species hormones are involved or there has been any possibility of amino acid modification.

Work continues on parathyroid hormone (PTH), although the entire amino acid sequence has not as yet been completed. Data accumulated so far indicate that the biological activity may be centered near the amino end of the 83-amino acid chain,[99] and that with this hormone, like CT, the sites for biological activity and antigenicity reside in different parts of the chain.[99,100]

The biological activity of both CT and PTH appears to be mediated by cyclic AMP, both in kidney and bone.[83,101] In kidney, these hormones affect primarily the adenyl cyclase of the cortex, while vasopressin stimulates adenyl cyclase of renal medulla. Both these hormones appear to be important in the fine regulation of blood calcium. Another very important aspect of overall calcium balance is absorption from the gastrointestinal tract, which is primarily regulated by vitamin D. A significant recent development in this area is the discovery that vitamin D_3 is metabolized to 25-hydroxycholecalciferol,[102] which appears to be the active substance regulating the biosynthesis of the calcium-binding protein needed for calcium absorption.[103]

HORMONES OF GLUCOSE METABOLISM

Progress in the insulin field has been rapid in these two years, particularly in the area of the biosynthesis of the hormone. It is now well established that insulin, which has two peptide chains (A, 21 amino acids and B, 30 amino acids) cross-linked by two disulfide bridges, is synthesized as a single peptide chain, proinsulin, in which the A and B chains of insulin are connected by a "connecting peptide" (C-peptide) chain of 33 (porcine) or 30 (bovine) amino acids. Work on proinsulin has been reviewed,[104,105] and the amino acid sequences of bovine[104] and porcine[106] proinsulins have been published. The amino acid composition of cod proinsulin has also appeared.[107] Two different proinsulins have been demonstrated in the rat,[108] and proinsulin has been isolated from human islet cell tumor tissue cultures.[109] The structures of porcine and bovine proinsulins are as follows:

Porcine: H_2N-B chain-Arg-Arg-Glu-Ala-Gln-Asn-Pro-Gln-Ala-Gly-
Bovine: H_2N-B chain-Arg-Arg-Glu-Val-Glu-Gly-Pro-Gln-Val-Gly-

Porcine: -Ala-Val-Glu-Leu-Gly-Gly-Gly-Leu-Gly-Gly-Leu-Gln-Ala-
Bovine: -Ala-Leu-Glu-Leu-Ala-Gly-Gly-Pro-Gly-Gly————————

Porcine: -Leu-Ala-Leu-Glu-Gly-Pro-Pro-Gln-Lys-Arg-A chain-CO_2H
Bovine: -Gly-Ala-Leu-Glu-Gly-Pro-Pro-Gln-Lys-Arg-A chain-CO_2H

The unique feature of the proinsulin molecule is that its thermodynamically most stable conformation is one in which the cysteine residues of the insulin A and B chains are held so that the proper disulfide bridges may form to establish the A-chain loop and the two A-B cross-links. Following establishment of the proper disulfide bridges, the C-peptide is removed by an as yet unidentified enzyme, and the resulting insulin and C-peptide are secreted from the β-cell granules together.[110,111] It is possible that this cleavage may be mediated *in vivo* by the combined action of trypsin and carboxypeptidase B, since the C-peptide as isolated from tissue lacks the C-terminal basic residues.[110] *In vitro*, the action of trypsin on proinsulin yields des-Ala-insulin (the C-terminal alanine is removed from the B chain). If conversion to insulin is blocked, it can be demonstrated that proinsulin has less than 5% insulin activity in any assay.

Proinsulin antibodies have been prepared in several laboratories, and have been used to show that when the disulfide bridges of proinsulin are broken by reduction in urea solution they can be reformed in the proper order in good yield by reoxidation. This is in contrast to insulin, where reduction and reoxidation, unless done under very special conditions lead to poor recovery of the correct molecule, due to randomization of the disulfide bridges. The presence of proinsulin in serum has been demonstrated by immunoassay techniques.[112,113] This procedure is complicated by the fact that homologous insulin, proinsulin, and C-peptide cross-react. More accurate values for proinsulin were obtained by removal of insulin antibodies by adsorption on insulin-Sephadex. Insulin-Sepharose has also been used for purification of insulin antibodies.[114] In contrast to these findings, bovine proinsulin antibodies have been reported [115] not to cross-react strongly with human or porcine proinsulin.

Amino acid sequences for several fish insulins have been reported,[116,117] and chemical syntheses in the field continue to appear.[118,119] A most remarkable paper is that of Cuatrecasas, [120] who reported that insulin bound chemically to Sepharose beads by the cyanogen bromide method retained full biological activity in the isolated fat cell assay. The beads to which the insulin was bound were as large as the fat cells, and controls showed that the insulin did not penetrate the cells. The only tenable conclusion from these data is that the site of action of insulin on fat cells in on the exterior of the cell membrane. The effects of insulin and several other hormones on synthetic lipid membranes have been reported.[121]

The hyperglycemic pancreatic hormone *glucagon* continues to be the subject of active research; two reviews have appeared.[122,123] A total synthesis of the porcine hormone has been published.[124] The physiological role of glucagon is still not completely understood, particularly with respect to its inter-actions with insulin and other hormones. Part of the confusion has been due to the presence of a substance (enteroglucagon, or "cross-reacting material") produced by the small intestine during glucose absorption.[125,126]

This material appears to differ in molecular size from glucagon and is not hyperglycemic, but cross-reacts with glucagon in immunoassays. Glucagon is useful clinically as a diagnostic agent for pheochromocytoma[127] and insulinoma,[128] and shows promise as a cardiotonic agent because of its inotropic and chronotropic effects.[129-131] A simplified, rapid immunoassay for glucagon has been described.[132]

HORMONES OF THE DIGESTIVE SYSTEM

The last two years have seen a great deal of activity in this field which remains confusing because of the great overlap in biological activities of many of the hormones and the difficulty of obtaining adequate supplies of pure material for accurate chemical studies.

Recent work on gastrin, the peptide which stimulates production of gastric acid and pancreatic enzymes, has been covered in two conferences[133,134] and two reviews.[135,136] Amino acid sequences are known for the human, porcine,[137] bovine (the same as ovine),[138] canine,[139] and feline[140] hormones. Syntheses for all but the feline peptide have been reported. All are 17-residue peptides having the amino end blocked by a pyrrolidone carboxylic acid (PCA, pyroglutamic acid) residue, and the carboxyl group blocked as the amide. The structures of these peptides, which differ by no more than two amino acids, are shown below:

Human:	PCA-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr(R)-Gly-Trp-Met-Asp-Phe-NH_2
Porcine:	——————Met——————
Canine:	——————Met——Ala——————
Feline:	——————Leu————Ala——————
Ovine-Bovine:	——————Val————Ala——————

The R group on the tyrosine hydroxyl can be hydrogen or sulfate. Only the C-terminal tetrapeptide amide is needed for full biological activity. Structure-activity relationships in the tetrapeptide have been studied in over 500 analogs.[137] A very active derivative, t-butyloxycarbonyl-β-Ala-Trp-Met-Asp-Phe-NH_2 (pentagastrin), has been much used in biological studies. New bioassays[141,142] and radioimmunoassays[143-145] have been developed. The action of gastrin is inhibited by secretin, cholecystokinin enterogastrone, angiotensin II, and a synthetic compound, 2-phenyl-2-(2-pyridyl)-thioacetamide.[134]

Cholecystokinin (CCK), which causes gallbladder contraction, and pancreozymin, which causes pancreatic enzyme secretion, are the same molecule. Jorpes[146] has reviewed the brilliant work he and Mutt have done in this area. Although the total sequence of CCK, a chain of 33 amino acids, is not yet known, the C-terminal dodecapeptide amide has been sequenced and synthesized.[147] The C-terminal heptapeptide amide is the minimum structure needed for full biological activity. The C-terminal octapeptide is eight times as active as the entire hormone.[148] Removal of the sulfate from the tyrosine reduces the activity 300 fold. Two peptides isolated from frog skins, caerulein and phyllocaerulein,[149] have partial sequences identical to that of CCK, and show the same biological activities. All of these peptides have the same C-terminal five amino

acids as gastrin, and show some gastrin-like action. However, they are potent inhibitors of the gastrin-induced secretion of acid by the stomach.[150] Structures of the peptides are compared below:

CCK: ———Ile-Ser-Asp-Arg-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

Caerulein: PCA-Gln————————Thr————————

Phyllocaerulein: PCA-Glu————————Thr————————

The crucial structural feature for differentiation of gastrin and CCK activity seems to be the insertion of the additional methionine or threonine residue between the tyrosine and glycine of the gastrin chain.

Secretin, the 28-residue peptide amide which stimulates the secretion of bicarbonate by the pancreas, has been synthesized.[151] Its structure is remarkable in that over half of its amino acid residues are identical to those of glucagon. While there is much similarity in the cardiovascular actions of secretin and glucagon, there are clear differences in the responses of different organs.[152] A radioimmunoassay has been developed.[153]

Enterogastrone, the intestinal hormone which inhibits gastric motility has been the subject of much controversy. Since secretin and CCK inhibit gastric motility,[154] some have thought that enterogastrone is merely a reflection of some aspects of the physiological response to other hormones. However, there are differences in some aspects of the response pattern,[155] and secretin-free enterogastrone preparations have been reported.[156,157]

ERYTHROPOIETIN

The synthesis of erythrocytes in the bone marrow is under humoral control. This control system reminds one of the renin-angiotensin system, in that a substance produced in the kidney acts upon a plasma protein to produce the active hormone. Erythropoietin (ESF) has been the subject of a conference [158] and several reviews.[159-161] The "renal erythropoietic factor" (REF), which can be extracted from kidney light mitochondria,[162] is apparently produced in response to renal hypoxia. Androgens and cobalt are also effective stimuli of ESF production.[163] Action of REF on plasma produces ESF, which appears to be a glycoprotein having a molecular weight about 60,000. ESF has been highly purified, but only minute amounts of this material have been obtained. Effects of ESF on bone marrow heme,[164] RNA [165] and hemoglobin syntheses,[166] as well as on accumulation of iron,[167] have been published. A protein inhibitor of erythropoiesis has been described.[168]

ANGIOTENSIN

Although the flow of renin-angiotensin papers continues unabated, there still remain many unanswered questions about the details of the system and its physiological significance. A book on high blood pressure has appeared.[169] A large part of the problem with renin, the enzyme produced in kidney which acts upon the plasma globulin "renin substrate" to produce the decapeptide angiotensin I, has to do with suitable assay techniques. Although new biological,[170] chemical,[171] and immunological[172] assays have appeared, it is by no means evident that a real solution is at hand. One problem is the multiplicity of "renins." Skeggs' group has described [173] a new human kidney enzyme, pseudorenin, which does not

hydrolize human globulin substrate in plasma but does hydrolyze hog globulin substrate and the tetradecapeptide substrate (a peptic fragment of the globulin substrate which contains the angiotensin sequence). Renin-like enzymes have been described in other tissues, e.g., pregnant uterus and its venous effluent.[174,175] The renin substrate in hog plasma has been found to exist in at least five different forms, and the substrate specificity of renin, while it has been studied,[176] is not completely understood.

It appears from recent work that most of the enzymes which convert the inactive angiotensin I to the active octapeptide angiotensin II (converting enzymes), are localized in lung [79,177-179] and other tissue beds,[180,181] rather than in plasma, as previously thought. The pulmonary converting enzyme is inhibited by "bradykinin potentiating factor," a group of small peptides from snake venom.[182] Immunoassays for angiotensin I [183] and angiotensin II [184] have appeared. "Substance A" has been identified as angiotensin II,[185] and "pepsitensin" as angiotensinyl(1)-leucine.[186] New evidence on the role of angiotensin in the aldosterone-mediated regulation of plasma sodium,[187,188] in renovascular hypertension,[189] in contraceptive therapy,[190] and as a possible adrenergic neuro-transmitter[191] has been published. Removal by various tissue beds can apparently account for all the destruction of angiotensins.[192,193] Angiotensin II analogs,[194] angiotensin I [195] and the tetradecapeptide substrate[173] have been synthesized by the solid phase method. The imidazole of the histidine in angiotensin II can be replaced by a pyrazole ring with retention of activity,[196] but not by a thiazole.[197]

THE KININS

Summaries of work in this field have appeared as two reviews,[198,199] the proceedings of two symposia,[200,201] and abstracts of a third.[202] Characteristics of pancreatic kallikrein [203] and its subcellular location [204] have been reported. The possible presence of labile ester bonds in the plasma globulin kininogen continues to be studied.[205] Plasmin has been shown to liberate methionyl-lysyl-bradykinin from kininogen II,[206] and an immunoassay for kininogen has been developed.[207] Useful immunoassays for bradykinin (BK) have been difficult to obtain, but two new procedures have recently appeared,[208,209] as well as new information on the structural requirements for binding of BK to antibodies.[210] New evidence has appeared on the roles of kinins in hemorrhagic [211] and endotoxin [212] shock, in pulmonary emphysema,[213] in anaphylaxis,[214] in the central nervous system [215,216] and in the reorganization of the fetal circulation at birth.[217] The mechanism of the very impressive pulmonary disappearance of BK [79] has been shown to be hydrolysis of the peptide by multiple peptidases located in the pulmonary capillaries.[218] The hypothesis of Stewart and Woolley concerning the relative importance of the amino (unimportant) and carboxyl (important) groups of BK for biological activity has been further confirmed by the syntheses of desamino BK (active) and descarboxyl BK (inactive).[219] BK has been synthesized by a "reversed solid phase" technique.[220] Habermann's kinin-yielding peptic peptides have been synthesized,[221] as well as all of the possible fragments of BK.[222] Polistes kinin, a peptide in wasp venom, has been sequenced [200] and synthesized.[223] This peptide is extremely active in increasing capillary permeability and producing pain, and is completely resistant to the pulmonary kininases. A new kinin has been obtained by the

action of enzymes from polymorphonuclear (PMN) leucocytes on kininogens.[224] PMN kinin-forming enzymes are probably important in inflammation, and show unusual patterns of behavior toward trasylol and other inhibitors.

Substance P (SP), a group of kinin-like peptides isolated from brain and intestinal mucosa, shows pharmacological properties most closely related to eledoisin and physalaemin, and may be chemically related to them. Like them, it stimulates salivation;[225] this may be a true endocrine action. Recent papers have described preparation of SP peptides from bovine brain[226] and peripheral nerve,[227] as well as preliminary structural work.[228]

REFERENCES

1. N. Back.,L. Martini and R. Paoletti, eds., "Pharmacology of Hormonal Polypeptides and Proteins," Plenum Publishing Corp., 1968.
2. R. I. Dorfman, "Methods in Hormone Research," 2d ed., Vol. 11A, Academic 1969.
3. R. W. Butcher, New England J. Med., 279, 1378 (1968).
4. J. M. Robson and R. S. Stacey, eds., "Recent Advances in Pharmacology," 4th ed., Little, Brown, 1968.
5. W. Haymaker, E. Anderson and W. Nauta, eds., "The Hypothalamus, C. C. Thomas, 1969.
6. L. Martini, F. Fraschini and M. Motta, Recent Progr. Hormone Res., 24, 439 (1968).
7. A. V. Schally, H. Arimura, C. Y. Bowers, A. J. Kastin, S. Sawano and T. W. Redding, ibid., p. 497.
8. A. V. Schally and A. J. Kastin, Triangle (Sandoz) 9, 19 (1969).

8a. S. M. McCann and J. C. Porter, Physiol. Revs., 49, 240 (1969).

9. R. Burgus, T. F. Dunn and D. M. Desiderio, Compt. Rend. Acad. Sci., 269 1870 (1969).
10. K. Folkers, F. Enzmann and J. Boler, Biochem. Biophys. Res. Comm., 37, 123 (1969).
11. A. V. Schally, T. W. Redding, C. Y. Bowers and J. F. Barrett, J. Biol. Chem., 244, 4077 (1969).
12. A. V. Schally, S. Sawano, A. Arimura, J. F. Barrett, I. Wakabayashi and C. Y. Bowers, Endocrinol., 84, 1493 (1969).
13. E. F. Couch, A. Arimura, A. V. Schally, M. Saito and S. Sawano, Endocrinol., 85, 1084 (1969).
14. V. Zor, T. Kaneko, H. P. G. Schneider, S. M. McCann, I. P. Lowe, G. Bloom, B. Borland and J. B. Field, Proc. Nat. Acad. Sci, 63, 918(1969).
15. M. Sonenberg, ed., Ann. N. Y. Acad. Sci., 148, 289-571 (1968).
16. A. Pecile and E. E. Muller, eds., "Internat. Symp. on Growth Hormone," Milan, Excerpta Medica, 1968.
17. C. H. Li, J. S. Dixon and W-K. Liu, Arch. Biochem. Biophys., 133, 70 (1969).
18. J. B. Mills, Nature, 213, 631 (1967).
19. L. Oliver and A. S. Hartree, Biochem. J., 109, 19 (1968).
20. M. O. Dayhoff, "Atlas of Protein Sequence and Structure," 4, D-227(1969).
21. C. Pena, A. C. Paladini and J. M. Dellacha, Biochim. Biophys. Acta, 194, 320 (1969).

22. M. Sonnenberg, J. M. Dellacha, C. A. Free and A. C. Nadler, J. Endocrinol., 44, 255 (1969).
23. J. Brovetto-Cruz and C. H. Li, Biochem., 8, 4695 (1969); T. A. Bewley, J. Brovetto-Cruz and C. H. Li, ibid., p. 4701.
24. P. H. Henneman, J. Am. Med. Assoc., 205, 828 (1968).
25. C. H. Li, M. M. Grumbach, S. L. Kaplan, J. B. Josimovich, H. Friesen and K. J. Catt, Experientia, 24, 1288 (1968).
26. H. G. Friesen, S. Suwa and P. Pare, Recent Prog. Hormone Res., 25, 161 (1969).
27. H. J. Quabbe, Z. Klin. Chem., 7, 259 (1969).
28. H. S. Jacobs, J. Clin. Path., 22, 710 (1969).
29. K. Sundaram and M. Sonenberg, J. Endocrinol., 44, 517 (1969).
30. M. Moloney and J. G. Devlin, Irish J. Med. Sci., 8, 469 (1969).
31. L. A. Frohman, L. L. Bernardis and K. J. Kant, Science, 162, 580 (1968).
32. J. P. MacManus and J. F. Whitfield, Proc. Soc. Exp. Biol. Med., 132, 409 (1969).
33. C. S. Nicoll, J. A. Parsons and R. P. Fiorindo, J. Endocrinol., 45, 183 (1969).
34. C. H. Li, J. S. Dixon, T-B. Lo, Y. A. Pankov and K. D. Schmidt, Nature, 224, 695 (1969).
35. S. Ellis, R. E. Grindeland, J. M. Nuenke and P. X. Callahan, Endocrinol., 85, 886 (1969).
36. Y. Amenomori, C. L. Chen and J. Meites, Endocrinol., 86, 506 (1970).
37. H. Nagasawa, C. L. Chen and J. Meites, Proc. Soc. Exp. Biol. Med., 132, 859 (1969).
38. E. Rosemberg, ed., "Gonadotropins 1968," (Proceedings, Workshop Conference held at Vista Herosa, Mor., Mexico, June, 1968), Los Altos, Calif. Geron-X Inc., 1968.
39. "Reproductive Endocrinology," Brit. Med. Bull., 26, No. 1 (1970).
40. M. Fujino, W. M. Lamkin, J. D. Mayfield, and D. N. Ward, Gen. Comp. Endocrinol., 11, 444 (1968).
41. L. E. Richert, M. A. Rasco, D. N. Ward, G. D. Niswender and A. R. Midgley, J. Biol. Chem., 244, 5110 (1969).
42. E. A. Diczfalusy, ed., "Immunoassay of Gonadotropins," Karolinska Symposia, 1969.
43. R. B. Jaffe and A. R. Midgley, Jr., Obstet. Gynec. Survey, 24, 200(1969).
44. B. Shome, D. M. Brown, S. M. Howard and J. G. Pierce, Arch. Biochem. Biophys., 126, 456 (1968).
45. S. M. Howard and J. G. Pierce, J. Biol. Chem., 244, 6468 (1969).
46. W. D. Odell, L. Vaslager, C. Charters and R. Bates, Clin. Res., 16, 115 (1968).
47. T. Kaneko, U. Zor and J. B. Field, Science, 163, 1062 (1969).
48. M. Fujino, C. Hatanaka and O. Nishimura, Chem. Pharm. Bull., 17, 2186 (1969).
49. G. I. Tesser and W. Rittel, Rec. Trav. Chim., 88, 553 (1969).
50. W. W. Downie, K. Whaley, M. A. Wright, M. A. Bell and A. K. Taylor, Brit. Med. J., 4, 487 (1968).
51. T. S. Danowski, K. Hofmann, F. A. Weigand and J. H. Sunder, J. Clin. Endocrinol., 28, 1120 (1968).
52. A. Walser and T. Mueller, Schweiz. Med. Wschr., 98, 717 (1968).
53. S. Berson and R. S. Yalow, J. Clin. Invest., 47, 2725 (1968).

54. J. J. Schnure, M. Reichlin and V. K. Vance, Clin. Res., 16, 115 (1968).
55. H. P. Bar and O. Hechter, Biochem. Biophys. Res. Comm., 35, 681 (1969).
56. H. Yajima, K. Kawasaki, H. Minami, H. Kawatani, N. Mizokami and Y. Okada, Biochim. Biophys. Acta, 175, 228 (1969).
57. H. Yajima, Y. Okada, Y. Kinomura, N. Mizokami and H. Kawatani, Chem. Pharm. Bull., 17, 1237 (1969).
58. B. Berde, ed., "Neurohypophyseal Hormones and Similar Polypeptides," Handbuch Exp. Pharmacol., Vol 23, Springer-Verlag, 1968.
59. H. Takashima, V. J. Hruby and V. du Vigneaud, J. Am. Chem. Soc., 92, 677 (1970).
60. E. Kasafirek, K. Eisler and J. Rudinger, Coll. Czech. Chem. Comm., 34, 2848 (1969).
61. J. Meienhofer and Y. Sano, J. Am. Chem. Soc., 90, 2996 (1968).
62. J. W. M. Baxter, T. C. Wuu, M. Manning and W. H. Sawyer, Experientia, 25, 1127 (1969).
63. H. C. Beyerman and R. A. in't Veld, Rec. Trav. Chim., 88, 1019 (1969).
64. H. Takashima, W. Fraefel and V. du Vigneaud, J. Am. Chem. Soc., 91, 6182 (1969).
65. R. T. Havran and V. du Vigneaud, Ibid., p. 2696.
66. W. H. Sawyer, T. C. Wuu, J. W. Baxter and J. M. Manning, Endocrinol., 85, 385 (1969).
67. R. Acher, J. Chauvet and M. T. Chauvet, Gen. Comp. Endocrinol., 13, 357 (1969).
68. N. Wilson and M. Smith, ibid., p. 412.
69. L. F. Johnson, I. L. Schwartz and R. Walter, Proc. Nat. Acad. Sci., 64, 1269 (1969).
70. S. Hase, T. Morikawa and S. Sakakibara, Experientia, 25, 1239 (1969).
71. A. Kobayashi, S. Hase, P. Kiyoi and S. Sakakibara, Bull. Chem. Soc. Jap., 42, 3491 (1969).
72. G. Flouret and V. du Vigneaud, J. Med. Chem., 12, 1035 (1969)
73. G. W. Bisset and B. J. Clark, Nature, 218, 197 (1968).
74. W. Fraefel and V. du Vigneaud, J. Am. Chem. Soc., 92, 1030 (1970).
75. T. Dousa, O. Hechter, R. Walter and I. Schwartz, Science, 167, 1134 (1970).
76. M. Miller and A. M. Moses, Endocrinol., 84, 557 (1969).
77. W-H. Wu and J. H. Rockey, Biochem., 8, 2719 (1969).
78. P. Biron and J-C. Boileau, Can. J. Physiol. Pharmacol., 47, 713 (1969).
79. J. R. Vane, Brit. J. Pharmacol., 35, 209 (1969).
80. H. Sachs, P. Fawcett, Y. Takabatake and R. Portanova, Recent Progr. Hormone Res., 25, 447 (1969).
81. P. F. Hirsch and P. L. Munson, Physiol. Revs., 49, 548 (1969).
82. G. V. Foster and I. MacIntyre, in Robson and Stacey, eds., Recent Adv. in Pharmacol., 4th ed., Churchill, 1968, p. 247.
83. G. D. Aurbach, J. T. Potts, Jr., L. R. Chase and G. L. Melson, Ann. Intern. Med., 70, 1243 (1969).
84. R. H. Savage, Guys Hosp. Rep., 118, 433 (1969).
85. P. Sieber, M. Brugger, B. Kamber, B. Riniker and W. Rittel, Helv. Chim. Acta, 51, 2057 (1968).
86. B. Riniker, M. Brugger, B. Kamber, P. Sieber and W. Rittel, Helv. Chim. Acta, 52, 1058 (1969).
87. St. Guttmann, J. Pless, R. L. Huguenin, E. Sandrin, H. Bossert and K. Zehnder, ibid., p. 179.

88. H. B. Brewer and R. Ronan, Proc. Nat. Acad. Sci., 63, 940 (1969).
89. H. D. Niall, H. T. Keutmann, D. H. Copp and J. T. Potts, Jr., Proc. Nat. Acad. Sci., 64, 771 (1969).
90. R. Neher, B. Riniker, W. Rittel and H. Zuber, Helv. Chim. Acta., 51, 1900 (1968).
91. A. G. Pearse, J. Endocrinol., 45 (Suppl.), 13 (1969).
92. A. Caniggia, C. Gennari and M. Bencini, Helv. Med. Acta, 35, 96 (1969).
93. T. E. West, J. L. O'Riordan and A. D. Care, J. Endocrinol., 45, 495(1969).
94. G. V. Foster, New England J. Med., 279, 349 (1968).
95. M. B. Clark, G. W. Boyd, P. G. H. Byfield and G. V. Foster, Lancet, 1969 (11) 71.
96. R. M. Lequin, W. H. L. Hackeng and W. Schopman, J. Endocrinol., 44, 283 (1969).
97. F. M. Dietrich and W. Rittel, Nature, 225, 75 (1970).
98. R. M. Lequin, W. H. L. Hackeng, W. Schopman and A. D. Care, J. Endocrinol., 45, 309 (1969).
99. J. T. Potts, Jr., H. T. Keutmann, H. Niall, L. Deftos, H. B. Brewer and G. D. Aurbach, Excerpta Medica Intl. Congress Series No. 159 Proc. Third Parathyroid Conf., Montreal, 1967.
100. D. B. P. Goodman and H. Rasmussen, Biochim. Biophys. Acta, 153, 749(1968).
101. F. Murad, H. B. Brewer and M. Vaughan, Proc. Nat. Acad. Sci., 65, 446 (1970).
102. J. W. Blunt and H. F. DeLuca, Biochem., 8, 671 (1969).
103. J. G. Ebel, A. N. Taylor and R. H. Wasserman, Am. J. Clin. Nutr., 22, 431 (1969).
104. D. F. Steiner, J. L. Clark, C. Nolan, A. H. Rubenstein, E. Margoliash, B. Aten and P. E. Oyer, Recent Progr. Hormone Res., 25, 207 (1969).
105. A. H. Rubenstein and D. F. Steiner, Med. Clin. N. Amer, 54, 191 (1970).
106. R. E. Chance, E. M. Ellis and W. W. Bromer, Science, 161, 165 (1968).
107. P. T. Grant and K. B. M. Reid, Biochem. J., 110, 281 (1968).
108. J. L. Clark and D. F. Steiner, Proc. Nat. Acad. Sci., 62, 278 (1969).
109. D. F. Steiner and P. E. Oyer, Proc. Nat. Acad. Sci., 57, 473 (1967).
110. A. H. Rubenstein, J. L. Clark, F. Melani and D. F. Steiner, Nature, 224, 697 (1969).
111. J. L. Clark, S. Cho, A. H. Rubenstein and D. F. Steiner, Biochem. Biophys. Res. Comm., 35, 456 (1969).
112. C. C. Yip and J. Logothetopoulos, Proc. Nat. Acad. Sci., 62, 415 (1969).
113. A. M. Lawrence and L. Kirstens, Proc. Soc. Exp. Biol. Med., 131, 1142 (1969).
114. P. Cuatrecasas, Biochem. Biophys. Res. Comm., 35, 531 (1969).
115. A. H. Rubenstein, S. Cho and D. F. Steiner, J. Lab. Clin. Med., 72, 1010 (1968).
116. P. A. Neumann, M. Koldenhof and R. E. Humbel, Z. Physiol. Chem., 350, 1286 (1969).
117. P. Neumann and R. E. Humbel, Int. J. Protein Res., 1, 125 (1969).
118. H. Zahn, W. Danho, H. Klostermeyer, H. G. Gattner and J. Repin, Z. Naturforsch., 24B, 1127 (1969).
119. U. Weber, Z. Physiol. Chem., 350, 1421 (1969).
120. P. Cuatrecasas, Proc. Nat. Acad. Sci., 63, 450 (1969).
121. M. S. Kafka and C. Y. Pak, Biochim. Biophys. Acta, 193, 117 (1969).
122. A. M. Lawrence, Ann. Rev. Med., 20, 207 (1969).

123. A. M. Lawrence, Med. Clinics N. Amer., 54, 183 (1969).
124. E. Weunsch, E. Jaeger and R. Scharf, Chem. Ber., 101, 3664 (1968).
125. R. H. Unger, A. Ohneda, I. Valverde, A. M. Eisentraut and J. Exton, J. Clin. Invest., 47, 48 (1968).
126. I. Valverde, D. Rigopoulou, J. Exton, A. Ohneda, A. Eisentraut and R. H. Unger, Am. J. Med. Sci., 255, 415 (1968).
127. S. G. Sheps and F. T. Maher, J. Am. Med. Assoc., 205, 895 (1968).
128. V. Marks and E. Samols, J. Clin. Path., 21, 346 (1968).
129. W. W. Parmley, G. Glick and E. H. Sonnenblick, New England J. Med., 279, 12 (1968).
130. J. W. Linhart, S. S. Barold, L. S. Cohen, F. J. Hildner and P. Samet, Clin. Res., 16, 238 (1968).
131. W. A. Mahon, J. E. Morch and S. W. Klein, Circulation, 38, VI-132(1968).
132. K. Nonaka and P. P. Foa, Proc. Soc. Exp. Biol. Med., 130, 330 (1969).
133. "A discussion on Polypeptide Hormones," Proc. Roy. Soc. (London), B170, 1 (1968).
134. Symposium on Gastrin, Federation Proc., 27, 1310-1344 (1968).
135. J. C. Thompson, Ann. Rev. Med., 20, 291 (1969).
136. R. M. Preshaw, in Robson and Stacey, eds., Recent Adv. in Pharmacol., 4th ed., Churchill, 1968, p. 263.
137. G. W. Kenner and R. C. Sheppard, Proc. Roy. Soc. (London), B170, 89 (1968).
138. K. L. Agarwal, G. W. Kenner and R. C. Sheppard, J. Chem. Soc., 1969c, p. 954.
139. K. L. Agarwal, G. W. Kenner and R. C. Sheppard, Experientia, 25, 346 (1969).
140. K. L. Agarwal, G. W. Kenner and R. C. Sheppard, J. Am. Chem. Soc., 91, 3096 (1969).
141. D. D. Richardson, J. Physiol., 198, 613 (1968).
142. B. Wilken, M. D. Turner and J. D. Hardy, Clin. Res. 16, 39 (1968).
143. J. E. McGuigan and W. L. Trudeau, New England J. Med., 278, 1308 (1968).
144. J. Hansky and M. D. Cain, Lancet, 1969-11, 1388.
145. R. S. Yalow and S. A. Berson, Gastroenterology, 58, 1 (1970).
146. J. E. Jorpes, Gastroenterology, 55, 157 (1968).
147. M. A. Ondetti, J. Pluscec, E. F. Sabo, J. T. Sheehan and N. Williams, J. Am. Chem. Soc., 92, 195 (1970).
148. B. Rubin, S. L. Engel, A. M. Drungis, M. Dzelzkans, E. O. Grigas, M. H. Waugh and E. Yicas, J. Pharm. Sci., 58, 955 (1969).
149. G. Bertaccini, J. M. Cei, G. DeCaro, V. Erspamer and M. Impicciatore, Brit. J. Pharmocol., 37, 198 (1969).
150. A. M. Brooks, A. Agosti, G. Bertaccini and M. I. Grossman, New England J. Med., 282, 535 (1970).
151. A. Bodanszky, M. A. Ondetti, V. Mutt and M. Bodanszky, J. Am. Chem. Soc., 91, 944 (1969).
152. G. Ross, Brit. J. Pharmacol., 37, 528P (1969).
153. J. D. Young, L. Lazarus, D. J. Chrisholm and F. F. V. Atkinson, J. Nuclear Med., 9, 641 (1968).
154. K. Sugawara, J. Isaza, J. Curt and E. R. Woodward, Am. J. Physiol., 216, 1633 (1969).
155. D. F. Magee and S. Nakajima, Experientia, 25, 1051 (1969).

156. H. W. Lucien, Z. Itoh, D. C. Sun, J. Meyer, N. Carlton and A. V. Schally, Arch. Biochem. Biophys., 134, 180 (1969).
157. J. C. Brown, R. A. Pederson, E. Jorpes and V. Mutt, Canad. J. Physiol. Pharmacol., 47, 113 (1969).
158. J. W. Fisher, ed., Ann. N. Y. Acad. Sci., 149, 1-583 (1968).
159. S. B. Krantz, Med. Clin. N. Amer., 54, 173 (1970).
160. J. W. Adamson, J. Eschbach and C. A. Finch, Am. J. Med., 44, 725(1968).
161. B. Kubanck, Hormone and Metabolic Res., 1, 151 (1969).
162. E. D. Zanjani, A. S. Gordon, K. K. Wong and W. D. McLaurin, Proc. Soc. Exp. Biol. Med., 131, 1095 (1969).
163. W. Fried and T. Kilbridge, J. Lab. Clin. Med., 74, 623 (1969).
164. S. S. Bottomley and G. A. Smithee, ibid., p. 445
165. A. D. Pavlov, Biochim. Biophys. Acta, 195, 156 (1969).
166. J. A. Hunter and J. Paul, J. Embryol. Exp. Morph., 21, 361 (1969).
167. M. E. Hrinda and E. Goldwasser, Biochim. Biophys. Acta, 195, 165 (1969).
168. J. Plavis, W. A. Neal, R. R. Moores, E. Gardner, D. A. Alford, L. L. Smith, C-S. Wright and E. T. Welch, J. Lab. Clin. Med., 74, 608 (1969).
169. G. Pickering, "High Blood Pressure," Churchill, 1968.
170. J. W. Ryan, J. K. McKenzie and M. R. Lee, Biochem. J., 108, 679(1968).
171. A. Reinharz and M. Roth, Eur. J. Biochem., 7, 334 (1969).
172. G. W. Boyd, A. E. Fitz, A. R. Adamson and W. S. Peart, Lancet, 1969-1, 213

173. L. T. Skeggs, K. E. Lentz, J. R. Kahn, F. E. Dorer and M. Levine, Circulation Res., 25, 451 (1969).
174. J. W. Ryan and D. C. Johnson, Biochim. Biophys. Acta, 191, 386 (1969).
175. M. Oda and T. Fukuda, Tohoku J. Exp. Med., 97, 311 (1969).
176. L. T. Skeggs,K.E. Lentz, J. R. Kahn and H. Hochstrasser, J. Exp. Med., 128, 13 (1968).
177. P. Biron and C. G. Huggins, Life Sci., 7, 965 (1968).
178. Y. S. Bakhle, Nature, 220, 919 (1968).
179. D. W. Cushman and H. S. Cheung, Federation Proc., 28, 799 (1969).
180. M. Roth, A. F. Weitzman and Y. Piquilloud, Experientia, 25, 1247 (1969).
181. S. Oparil, C. A. Sanders and E. Haber, Federation Proc., 28, 580 (1969).
182. S. H. Ferreira, L. J. Greene, V. A. Alabaster, M. Bakhle and J. R. Vane, Nature, 225, 379 (1970).
183. H. J. G. Hollemans, J. Van der Meer and W. Kloosterziel, Clin. Chim. Acta, 24, 353 (1969).
184. M. C. Dain, K. J. Catt and J. P. Coghlan, Biochim. Biophys. Acta., 194, 322 (1969).
185. N. S. Thampi and C. G. Huggins, Biochem. Pharmacol., 17, 2018 (1968).
186. K. Hochstrasser, F. Bachhuber and E. Werle, Z. Physiol. Chem., 350, 1225 (1969).
187. W. W. Davis, L. R. Burwell and F. C. Bartter, Proc. Nat. Acad. Sci., 63, 718 (1969).
188. D. J. Gocke, J. Gerten, L. M. Sherwood and J. H. Laragh, Circulation Res., 24, (Suppl. 1) 1-131 (1969).
189. T. A. Kotchen, B. Lytton, L. B. Morrow, P. J. Mulrow, P. M. Shutkin and H. C. Stansel, Arch. Int. Med., 125, 265 (1970).
190. G. D. Lubash, R.H. Bard and J.A. Kline, Am. J. Med. Sci., 257, 155(1969).

191. D. Palaic and J-C. Panisset, Biochem. Pharmacol., 18, 2693 (1969).
192. W. P. Leary and J. G. Ledingham, Nature, 222, 959,(1969).
193. Y. S. Bakhle, A. M. Reynard and J. R. Vane, ibid, p. 222.
194. M. C. Khosla, N. C. Chaturvedi, R. R. Smeby and F. M. Bumpus, Biochemistry, 7, 3417 (1968).
195. N. S. Thampi, G. Schoellmann, M. W. Hurst and C. G. Huggins, Life Sci., 7, 641 (1968).
196. R. Andreatta and K. Hofmann, J. Am. Chem. Soc., 90, 7334 (1968).
197. N. C. Chaturvedi, W. K. Park, R. R. Smeby and F. M. Bumpus, Abstr. Papers, Am. Chem. Soc., 156th Meeting BIOL-187 (1968).
198. R. W. Kellermeyer and R. C. Graham, Jr., New England J. Med.,279, 754, 802, 859 (1968).
199. M. Schachter, Physiol. Revs., 49, 509 (1969).
200. Federation Proc., 27, 49-99 (1968).
201. U. Hamberg, E. Amundsen and P. Olsson, eds., Scand. J. Clin. Lab. Invest., 24, Suppl. 107 (1969).
202. Pharmacol. Res. Comm., 1, 127-211 (1969).
203. F. Fiedler and E. Werle, Eur. J. Biochem., 7, 27 (1968).
204. K. D. Bhoola, Biochem. Pharmacol., 18, 2279 (1969).
205. J. S. Morley, Biochem. Biophys. Res. Comm., 33, 841 (1968).
206. P. B. Henriques, E. Grapanhuk, N. Kauritcheva and P. Budnitskaya, Biochem. Pharmacol., 18, 1788 (1969).
207. J. Spragg, E. Haber and K.F. Austen, Federation Proc., 28, 363 (1969).
208. R. C. Talamo, E. Haber, K.F. Austen, J. Lab. Clin.Med., 74, 816(1969).
209. T. L. Goodfriend and D. L. Ball, J. Lab. Clin. Med., 75, 501 (1969).
210. J. Fischer, J. Spragg, R. C. Talamo, J. V. Pierce, K. Suzuki, K. F. Austen and E. Haber, Biochemistry, 8, 3750 (1969).
211. W. H. Massion, G. Blumel, L. Peschl and H. Rettenbacher-Daubner, Federation Proc., 28, 272 (1969).
212. W. A. Pettinger and R. Young, Federation Proc., 28, 799 (1969).
213. J. Lieberman, New England J. Med., 281, 279 (1969).
214. M. S. Starr and G. B. West, Brit. J. Pharmacol., 37, 178 (1969).
215. F. G. Graeff, I. R. Pela, and M. Rocha e Silva, Brit. J. Pharmacol., 37, 723 (1969).
216. L. Pearson, G.A. Lambert and W.J. Lang, Eur. J. Pharmacol., 8, 153(1969).
217. L. G. Eltherington, J. Stoff, T. Hughes and K. L. Melmon, Circulation Res., 22, 747, (1968).
218. J. W. Ryan, J. Roblero and J.M.Stewart, Biochem J., 110, 795(1968).
219. W. D. Johnson, H. D. Law and R. O. Studer, Experientia, 25, 573 (1969).
220. M. Fridkin, A. Patchornik and E. Katchalski, J. Am. Chem. Soc., 90, 2953 (1968).
221. E. Schroder, Experientia, 25, 1126 (1969).
222. K. Suzuki, T. Abiko and N. Endo, Chem. Pharm. Bull., 17, 1671 (1969).
223. J. M. Stewart, Federation Proc., 27, 534 (1968).
224. L. M. Greenbaum, R. Freer, J. Chang, G. Semente and K. Yamafuji, Brit. J. Pharmacol., 36, 623 (1969).
225. F. Lembeck and K. Starke, Arch. Pharmakol. Exp. Pathol., 259, 375(1968).
226. J. Baldauf, W. Dobek and G. Zetler, Arch. Pharmakol. Exp. Pathol., 264, 354 (1969).
227. S. Hori, Jap. J. Physiol., 18, 746 (1968).
228. S. Hori, ibid., p. 772.

Chapter 20. Non-steroidal Anti-inflammatory Agents

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Introduction - No new non-steroidal antirheumatic drug has been marketed in 1969. Various reviews[1-6] have appeared which cover a variety of aspects of inflammation such as the pathogenesis, etiology, and biochemical considerations. With the exception of gouty arthritis, the etiology and pathogenesis of rheumatic and arthritic diseases remain obscure. Studies of the possible role of infectious inductions and/or adverse immunological reactions continue with the hope of defining the trigger mechanism for the chronic and self-perpetuating inflammatory processes in man.

Pharmacological Methodology and Biochemical Considerations - The difficulty in finding relevant test models for anti-rheumatic agents is well known. The mechanism of adjuvant induced arthritis in rats has been studied further.[7,8] It has been proposed that the first stage consists of an immunological response to mycobacterial antigen (S) followed by activation of a latent virus.[9] Evidence has also been presented supporting the view that the antigen which induces adjuvant arthritis is a combination of mycobacterial wax D and a constituent peculiar to the tissues of the rat.[10] The effect of thyroidal hormones on adjuvant induced arthritis in rats has also been studied.[11] It has been reported that the serum sulfhydryl/disulfide interchange reaction is impaired in arthritic rats and restored to normal by treatment with anti-inflammatory agents.[12]

Mycoplasmal injections into swine and mice produce syndromes which approximate the symptoms seen in arthritic patients. These models are based on the theory of microbial infection as a trigger mechanism.[13] The non-pathogen Corynebacterium rubrum can induce adjuvant arthritis in rats.[14]

Under suitable experimental conditions all major non-steroidal anti-inflammatory drugs inhibit multiplication of different viruses in cell cultures.[15] The synthetic polyanionic pyran copolymer has been found to induce interferon and to inhibit adjuvant-induced arthritis in rats.[9] These data suggest that a virus may be implicated in the pathogenesis of adjuvant-induced polyarthritis.

It has been suggested that the constituents of the kinin system in synovial fluid of polyarthritic joints are mainly derived from plasma and not from invading leucocytes.[16] Rheumatoid arthritic joints show higher protein concentrations than normal. Further, hyaluronic acid becomes more dilute and more abundant due to an increase in the amount of synovial fluid.[17] Higher than normal levels of serotonin in the blood of arthritic patients have been demonstrated.[18] Total hemolytic complement activities

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are depressed in the synovial fluids of rheumatoid arthritic patients.[19] A new in vitro method for assaying immunosuppressant potency is based on the inhibition of spontaneous rosette-forming cells from mice.[20]

The Tourniquet Syndrome in the hind legs of the rat is a model that is receiving some consideration.[21] Evans blue-carrageenin induced pleural effusion has been suggested as a model for the assay of non-steroidal anti-inflammatory agents.[22]

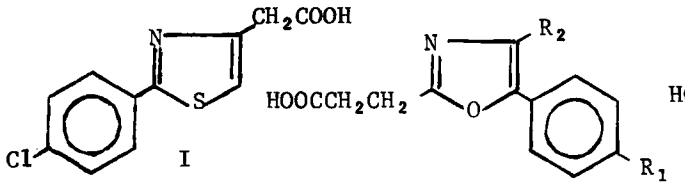
Advances in biochemistry and biochemical pharmacology have had considerable impact on many aspects of inflammation and arthritis. Recent studies cover membrane stabilization, enzyme inhibition, connective tissue metabolism, novel experimental models and viruses. Lysosomal membrane stabilization is considered an important mechanism of action of steroidal and non-steroidal anti-inflammatory drugs. Chloroquine, hydrocortisone, acetylsalicylic acid, phenylbutazone and niflumic acid stabilize isolated rat liver lysosomes at 37° in vitro, whereas gold, Imuran®, mefenamic acid and ibufenac exert no such effect.[23] At elevated temperatures (45°) anti-inflammatory drugs show no apparent stabilizing effect on isolated rat liver lysosomes.[24] Various steroidal anti-inflammatory drugs stabilize rabbit liver lysosomes in vitro.[25] The symptomatic relief of rheumatoid arthritis in pregnancy is well known. Human pregnancy serum is capable of stabilizing isolated lysosomes in vitro.[26] Non-steroidal anti-inflammatory drugs stabilize canine[27] and rabbit[28] erythrocytes against various modes of induced hemolysis in vitro. Inhibition of gelatin-induced erythrocyte aggregation is claimed to be a specific effect limited to anti-inflammatory drugs.[29] Mucopolysaccharide catabolism in adjuvant-induced arthritic rats is greater than normal and the intensity of the metabolism correlates directly with the severity of the arthritis.[30] Further, the anti-inflammatory effectiveness of steroids is shown to correlate with inhibition of mucopolysaccharide catabolism. Rumalon, a mucopolysaccharide peptide complex extracted from cartilage of calves, inhibits the activities of hyaluronidase, collagenase and papain.[31] Gold salts inhibit a variety of lysosomal enzymes obtained from the synovial fluids of rheumatoid arthritic patients[32] in whom the activities of such enzymes are markedly elevated.[16] Lysosomal enzymes are also markedly elevated in acute inflammations of the skin in man.[33] An acid pH optimum collagenase has been discovered in rat liver lysosomes which is inhibited in vitro by phenylbutazone and ibufenac.[34] The presence of synovial collagenase is not indicative of rheumatoid arthritis.[35] Various non-steroidal anti-inflammatory agents inhibit the release of lactic dehydrogenase and acid phosphatase from thrombin-aggregated platelets and inhibit hypotonic/hyperthermic lysis of erythrocytes in vitro.[36]

The beneficial effects of gold therapy[37] have led to new studies pertaining to its possible mechanism of action. Studies on the distribution of gold salts have been carried out. The transport of gold by the blood has been studied in rabbits and reveals an almost specific association with serum albumin.[38] Recent studies suggest that one mechanism of anti-inflammatory drugs involves the binding of free SH groups in vitro and in vivo.[39] Cysteine has been found to reverse the anti-inflammatory

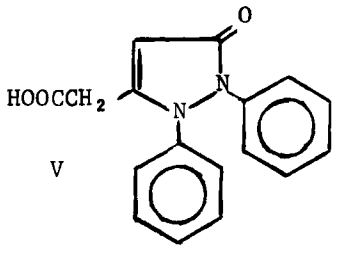
actions of acetylsalicylic acid, indomethacin, N-ethylmaleimide and ethacrynic acid tested in the cotton granuloma model using rats.[40] Gold salts inhibit the in vitro activity of cathepsin, acid phosphatase and β-glucuronidase obtained from human synovial fluid. It has been proposed that the enzyme inhibition by gold salts may occur through the binding of SH groups.[41]

Elevated activity of xanthine oxidase has been reported in livers of patients suffering from primary gout.[42] These data strengthen the contention that increased xanthine oxidase activity represents a primary biochemical lesion in gout. Trasylol, a trypsin-kallikrein inhibitor, exerts an anti-inflammatory effect against acute urate-induced arthritis in rabbits.[43] Filipin, a potent lysosomal membrane labilizer both in vitro and in vivo, is known to induce chronic degenerative joint diseases in rabbits. Filipin, digitonin and saponin injected into the hind paws of rats induce dose-related inflammatory responses and fevers.[44] Local administration of annanase, a proteolytic enzyme derived from the pineapple, to rabbit knee joints induces an osteoarthritis-simulated degenerative joint disease.[45]

New and Experimental Agents - Arylalkanoic Acids - Considerably more clinical data[46-48] is now available for fenclozic acid (I) (Myalex®, I.C.I. 54,450). The agent appears to have potency similar to phenylbutazone in acute screens and is more potent in chronic tests. The related compound (II) (Wy 21,743) is active in the anti-carrageenin and granuloma pouch screens and is claimed to be non ulcerogenic.[50] Activity in the U.V. erythema test has been reported for the acids (III)[51], (IV)[52], and (V).[53]

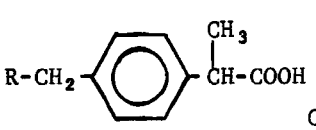


I II; R_1=H; R_2=C_6H_5 III; R_1=Br; R_2=H IV



V

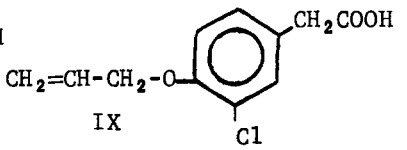
Ibuprofen (VI), Brufen®, is twenty times more potent than acetylsalicylic acid in the U.V. erythema assay.[54] Unlike ibufenac, no long-term toxicity has been reported for (VI)[55] and it is effective in man against rheumatoid arthritis but not osteoarthritis.[56] Studies in man indicate a half-life of six hours. The main metabolites are (VII) and (VIII), both of which are devoid of activity.[57] 4-Allyloxy-3-chlorophenylacetic acid (IX) (Mervan®) and its ethanolamine salt have been reported active in the clinic.[58]



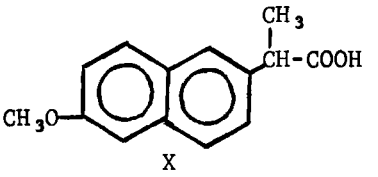
VI; R = $(CH_3)_2CH-$

VII; R = $(CH_3)_2C(OH)-$

VIII; R = $HOOC-CH(CH_3)-$

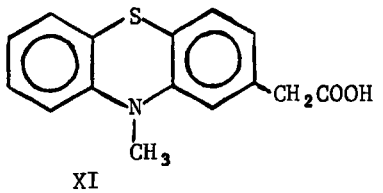


IX

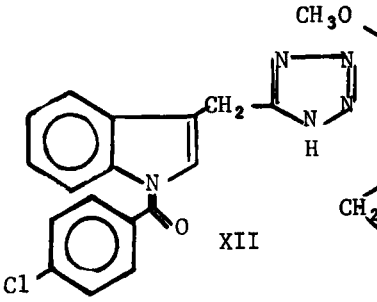


X

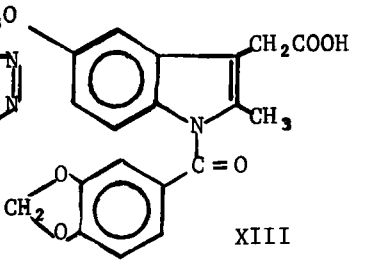
p-(n-Butoxy)-phenylacethydroxamic acid (Droxaryl®) has been reported to be effective in osteoarthritis and rheumatoid arthritis.[59,60] Potentiation of hydroxycortisone has been postulated as the mode of action.[61] The most active of a series of 6-substituted 2-naphthylacetic acids, (X) (d-isomer), is presently in clinical trial.[62] Structure activity relationships,[63] detailed pharmacology,[64] toxicological[65] and metabolic studies[66] for metiazinic acid (Soripal®, 16,091-RP) (XI) have been reported. In rabbits and man hydroxylated derivatives (free and conjugated) have been identified as metabolites but in dogs metiazinic acid S-oxide is the main metabolite.[66] (XI) has been reported to be clinically effective in rheumatoid arthritis and to be an effective analgesic.[67]



XI



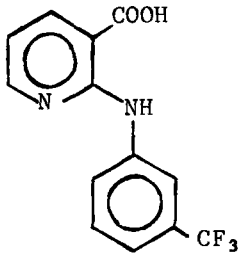
XII



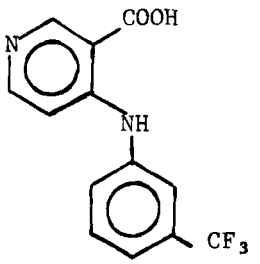
XIII

Indomethacin Related Agents - Structure activity relationships of tetrazole analogs of indomethacin have been published. The most active compound in the series is intrazole (XII; BLR 743),[68] which has been reported to be clinically effective in rheumatoid arthritis.[69] The methylenedioxy-analog (XIII) of indomethacin shows a spectrum of activity similar to oxyphenbutazone.[70] Glucuronides of indomethacin are claimed to have anti-inflammatory activity.[71,72]

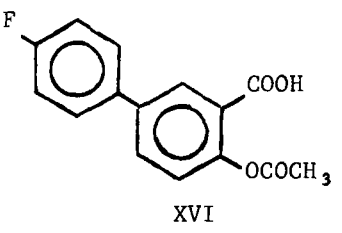
Fenamates and Salicylates - The synthesis[73] of radioactive niflumic acid (XIV) and metabolic studies in rats[74] and rabbits[75] have been reported and 2-aminonicotinic acid has been identified as a metabolite. Clinical studies with (XIV) indicate good gastric tolerance.[76] It is interesting to note that the isomer (XV) is a potent diuretic agent.[77] Structure activity relationships in the series of N-phenylanthranilic acids have been published.[78]



XIV



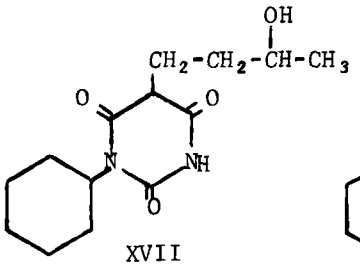
XV



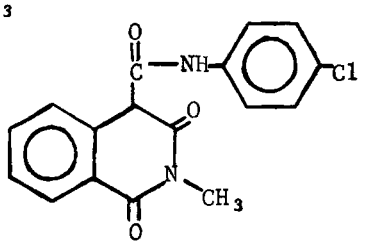
XVI

Flufenisal (XVI) is in clinical trial.[79,80]

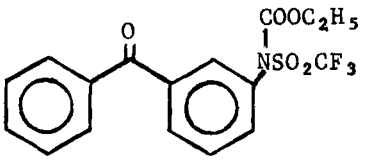
Miscellaneous - The main metabolite of paramidine (Bucolome®) has been identified as (XVII).[81] Structure activity relationships in this series have been published.[82] Anti-inflammatory activity has been reported for a series of acidic dioxo-isoquinoline-4-carboxanilides. The most potent in pharmacological tests is (XVIII) and members of the series are in clinical trial.[83] Triflumidate (XIX) and diflumidone sodium (XX) are similar to phenylbutazone in acute and chronic screens and are in clinical trial.[84]



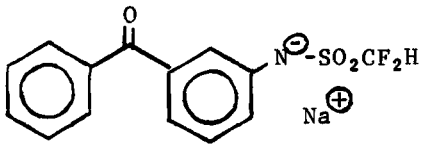
XVII



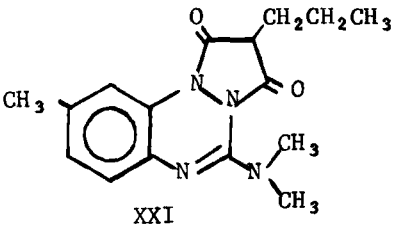
XVIII



XIX

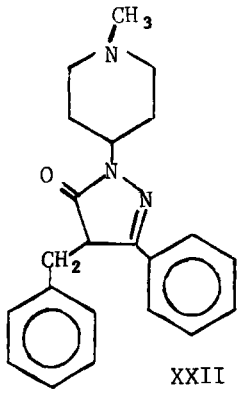


XX

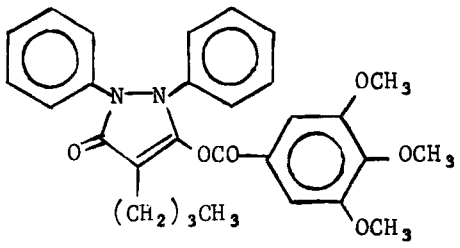


XXI

Additional reports have appeared on pharmacological and clinical investigations with azapropazone (XXI) (Apazone®).[85] Benzpiperylon[86] (XXII) and LH-150[87] (XXIII) are reported clinically effective. Trimethazone (XXIV) is reported as effective as phenylbutazone clinically and claimed to cause less gastric irritation or water retention.[88]



XXII



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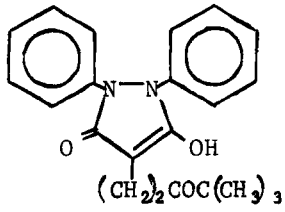
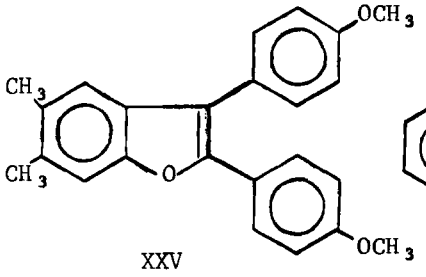
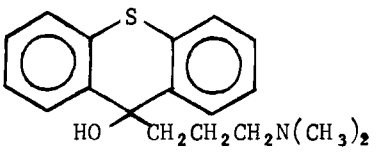


XXIV

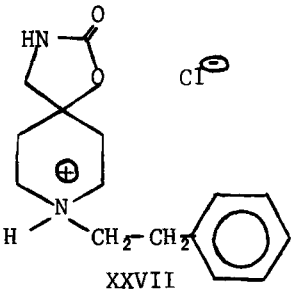
On the basis of ten double-blind clinical studies, benzydamine is considered as effective as oxyphenbutazone for the treatment of inflammatory edema.[89] Tribenoside (Glyvenol®) is in clinical trial in the U.S. for its anti-varicose effect; no effect has been seen on the articular index in cases of rheumatoid arthritis.[90] α,4-Dinicotinoyloxy-3-methoxy-acetophenone has prolonged anti-inflammatory activity in some pharmacological tests. β-Piperidino-propiophenones are also reported active.[91] Histidine is reported of value clinically in the treatment of rheumatoid arthritis.[92] Prolonged treatment with penicillamine is reported effective against rheumatoid arthritis[93] and a decline in rheumatoid factor is seen.[94] Sulfhydryl binding compounds such as N-ethylmaleimide and the diuretic ethacrynic acid are reported to have anti-inflammatory activity.[40] Glucosamine derivatives are considered effective against human osteoarthritis on intra-or periarticular administration.[95] Orgotein, a divalent metal chelate of a low molecular weight protein is being investigated clinically.[96] Several proteinases (bromelain, kinonase A_1, A_3, B_1) suppress carrageenin induced edema.[97] Livingston lysate (a human placental extract) is reported to give significant improvement in both osteo and rheumatoid arthritis.[98] Terpenes such as β-amyrin and bisabolol (a constituent of chamomile) are claimed to possess anti-inflammatory properties.[99] The alkaloids tomatine,[100] thalsimine, and dihydrothalsimine[101] have anti-inflammatory activity in experimental models. The anti-inflammatory use of the saponin escin[102] has been reviewed.[103] Some clinical efficacy is claimed for Vitamin K_1 in rheumatoid arthritis.[104] Further work has been reported on the possible anti-inflammatory activity of thiamine derivatives such as thiamine tetrahydrofurfuryl disulfide hydrochloride.[105] Certain sulfated polysaccharides appear to act as anti-inflammatory agents by interference with the kininogen inflammatory system.[106] 5-Acetyl-substituted indoxole and the benzofuran (XXV) are active in pharmacological tests.[107] Meprotixol (XXVI) is reported effective against rheumatoid arthritis in a double blind clinical study.[108]



XXV



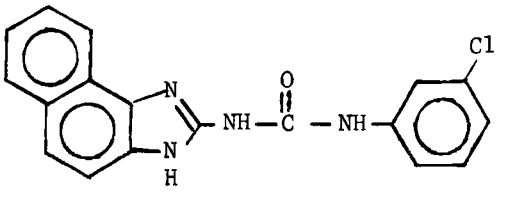
XXVI



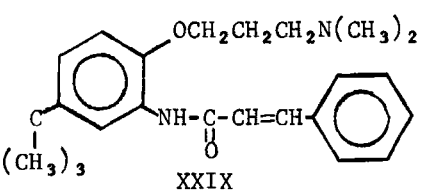
XXVII

Allopurinol (Zyloprim®) continues to be the recommended agent for the treatment of gout. Long term use of allopurinol results in minimal toxicity and no development of tolerance.[109] The action of thiopurinol on urinary and plasma uric acid has been investigated further.[110]

Immunosuppressive Agents - Cyclophosphamide holds promise for the treatment of rheumatoid arthritis.[111] It is effective in reversing the course of established experimental allergic encephalomyelitis.[112] Further reports have shown the clinical effectiveness of azathioprin (Imuran®) on prolonged administration for the treatment of rheumatoid arthritis.[113-115] Imuran® is also effective in patients with systemic lupus erythematosus.[116] Tris(chloroethyl)amine, an immunosuppressant, has shown protection against adjuvant arthritis in the rat.[117] Anti-lymphocyte serum has been studied repeatedly and its immunosuppressive and anti-inflammatory properties are interesting.[118,119] Spiro-oxazolidones, such as the bronchodilator fenspiride (XXVII) are reported to possess anti-inflammatory and immunosuppressive properties.[120] Ureas, such as (XXVIII) are reported as potent immunosuppressive agents.[121] The cinnamanilide (XXIX) appears to be a more specific immunosuppressive, anti-inflammatory agent than cinanserin.[122] Adamantoyl cytarabine (U 26,516) is superior to cytarabine[123] (Cytosar®) as an immunosuppressive agent.[124]



XXVIII



XXIX

Derivatives of podophyllin have been shown to have immunosuppressive and anti-inflammatory properties.[125]

References

1. K. J. Doebel, "The Chemistry of Non-Steroidal Anti-inflammatory Agents," Pure and Applied Chemistry, 19, 49-75 (1969).
2. W. S. C. Copeman, "Textbook of the Rheumatic Diseases," 4th Ed., 1969, Livingstone, London.
3. R. E. Brummett, "Non-steroid Anti-inflammatory Drugs," Pharm. Index, 11, 4 (1969).
4. "Conference on Rheumatoid Factors and Their Biological Significance," Ann. N. Y. Acad. Sci., 168, 1 (1969).
5. P. Schmied, P. Van Rossum and G. H. Fallet, "Traitement conservateur des rhumatismes inflammatoires chroniques," Schweiz. Med. Wochschr., 99, 933 (1969).
6. L. E. Ward, "Non-steroid Anti-inflammatory Agents in the Therapy of Rheumatic Diseases," J. Am. Geriat. Soc., 16, 523 (1968).
7. G. P. Velo, Boll. Soc. Ital. Biol. Sper., 45, 38 (1969).
8. M. Mikolajew, Z. Kuratowska, M. Kossakowska, M. Plachecka, M. Kopec, Ann. Rheum. Dis., 28, 35 (1969).
9. M. A. Kupusta, J. Mendelson, Arthr. Rheum., 12, 463 (1969).
10. C. M. Pearson and F. D. Wood, Immunology, 16, 157 (1969).
11. B. Steinetz, T. Giannini, M. Butler, F. Popick, Proc. Soc. Exptl. Biol. Med., 133, 401 (1970).
12. M. Butler, T. Giannini, D. Cargill, F. Popick, B. Steinetz, Proc. Soc. Exptl. Biol. Med., 132, 484 (1969).
13. C. H. Lack, Med. Clin. N. Am., 52, 667 (1968).
14. F. Paronetto, Proc. Soc. Exptl. Biol. Med., 133, 296 (1970).
15. A. D. Inglot, J. Gen.Virol., 4 203 (1969).
16. M. K. Jasani, M. Katori, G. P. Lewis, Ann. Rheum. Dis., 28, 497 (1969).
17. J. R. E. Fraser, G. S. Harris, B. J. Clarris, Ann. Rheum. Dis., 28, 419 (1969).
18. S. Stutts, O. B. Gum, Arthr. Rheum., 12, 336 (1969).
19. S. Ruddy, M. C. Britton, P. H. Schur, K. F. Austen, Arthr, Rheum., 12, 328 (1969).
20. J. F. Bach, M. Dardenne, C. Fournier, Nature 222, 998 (1969).
21. F. W. Eigler, W. Stock, I. Hofer, Z. Gesamte Exp. Med., 151, 55 (1969).
22. L. F. Sancilio, J. Pharmacol. Exptl. Therap., 168, 199 (1969).
23. L. J. Ignarro, Fed. Proc., 29, 784 (1970).
24. J. H. Brown, N. L. Schwartz, Proc. Soc. Exptl. Biol. Med., 131, 614 (1969).
25. A. M. Symons, D. A. Lewis, R. J. Ancill, Biochem. Pharmacol., 18, 2581 (1969).
26. K. H. Hempel, L. A. Fernandez, R. H. Persellin, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 386.
27. Y. Mizushima, S. Sakai, J. Pharm. Pharmacol., 21, 327 (1969).
28. B. Catanese, R. Lisciani, D. Piccinelli, Biochem. Pharmacol., 18, 1707 (1969).
29. P. Görög, I. B. Kovacs, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 769.
30. J. Gietka, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 729.
31. W. Weigel, P. Iwangoff, B. Jasinsky, Arzneim-Forsch., 19, 780 (1969).

32. S. Paltemaa, Acta Rheumatol. Scand., 14, 161 (1968).
33. P. Jansa, J. Kratky, Cesk. Patol., 5, 58 (1969).
34. A. J. Anderson, Biochem. J., 113, 457 (1969).
35. E. D. Harris, G. L. Cohen, S. M. Krane, Arthr. Rheum., 12, 92 (1969).
36. E. M. Glenn, B. J. Bowman, Proc. Soc. Exptl. Biol. Med., 130, 1327 (1969).
37. R. Hunger, Z. Gesamte Inn. Med., 24, 237 (1969).
38. E. G. McQueen, P. W. Dykes, Ann. Rheum. Dis., 28, 437 (1969).
39. M. W. Whitehouse, Arthr. Rheum., 12, 342 (1969).
40. A. L. Oronsky, L. Triner, O. S. Steinsland, G. G. Nahas, Nature 223, 619 (1969).
41. R. S. Ennis, J. L. Granda, A. S. Posner, Arthr. Rheum., 11, 756 (1968).
42. A. Carcassi, R. Marcolongo, E. Marinello, G. Riario-Sforza, C. Boggiano, Arthr. Rheum., 12, 17 (1969).
43. I. Spilberg, C. K. Osterland, Arthr. Rheum., 12, 334 (1969).
44. E. M. Glenn, S. C. Lyster, N. A. Rohloff, Proc. Soc. Exptl. Biol. Med., 130, 110 (1969).
45. G. C. Prasad, R. H. Singh, Indian J. Med. Res., 57, 1095 (1969).
46. T. M. Chalmers, J. E. F. Pohl, D. S. Platt, Ann. Rheum. Dis., 28, 590 (1969); T. M. Chalmers, J. H. Kellgren, D. S. Platt, Ann. Rheum. Dis., 28, 595 (1969); T. M. Chalmers, D. S. Platt, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 322.
47. A. Brees, ibid., Abstr. No. 321.
48. F. Dudley Hart, ibid., Abstr. No. 323.
49. B. B. Newbould. Brit. J. Pharmacol., 35, 487 (1969).
W. Hepworth, B. B. Newbould, G. J. Stacey, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 319.
50. M. E. Rosenthale, A. J. Begany, J. Malis, N. H. Grant, Fed. Proc., 28, Abstr. No. 554 (1969).
51. F. W. Short, L. M. Long, J. Heterocycl. Chem., 6, 707 (1969).
52. F. W. Short, G. M. Lockwood, J. Heterocycl. Chem., 6, 713 (1969).
53. F. W. Short, E. J. Schoeb, J. Heterocycl. Chem., 6, 723 (1969).
54. S. S. Adams, K. F. McCullough, J. S. Nicholson, Arch. Intern. Pharmacodyn., 178, 115 (1969).
55. T. M. Chalmers, Ann. Rheum. Dis., 28, 513 (1969).
56. N. Cardoe, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 318.
57. S. S. Adams, E. E. Cliffe, R. F. N. Mills, J. S. Nicholson, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 750.
58. N. P. Buu-Hoi, G. Lambelin, C. L. Gillet, J. Roba, M. Staquet, Naturwissenschaften, 56, 330 (1969).
G. Lambelin, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 314.
59. H. van Cauwenberge, P. Franchimont, J. Belg. Rhum. Med. Phys., 23, 133 (1968).
60. B. S. Rose, I. C. Isdale, P. W. Conlon, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 309.
61. J. Roba, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 310.
62. I. T. Harrison, B. Lewis, P. Nelson, W. Rooks, A. Roszkowski, A. Tomolonis, J. H. Fried, J. Med. Chem, 13, 203 (1970).
63. M. Messer, D. Farge, J. C. Guyonnet, C. Jeanmart, L. Julou, Arzneim.-Forsch., 19, 1193 (1969).

64. L. Julou, J. C. Guyonnet, R. Ducrot, M. C. Bardone, J. Y. Detaile, B. Laffargue, Arzneim.-Forsch., 19, 1198 (1969).
65. L. Julou, R. Ducrot, J. Fournel, P. Ganter, P. Populaire, J. Durel, J. Myou, S. Pascal, J. Pasquet, Arzneim.-Forsch., 19, 1207 (1969).
66. P. Populaire, B. Terlain, S. Pascal, G. Labreton, B. Decouvelacre, Arzneim.-Forsch., 19, 1214 (1969).
67. L. Volle, M. Depont, A. Frairot, C. Girerd, C. Lardet, 12th Intl. Rheum. Congress (Prague), 1969, Abst. No. 847.
68. P. F. Juby, T. W. Hudyma, J. Med. Chem., 12, 396 (1969). Council on Drugs, J. Am. Med. Assoc., 211, 819 (1970). J. S. Fleming, M. E. Bierwagen, A. W. Pircio, M. H. Pindell, Arch. Intern. Pharmacodyn., 178, 423 (1969).
69. D. W. Englund, S. H. Roth, 12th Intl. Rheum. Congress (Prague), 1969, Abst. No. 807; M. Rakic, L. Gross, K. Agre, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 325; D. W. Englund, S. H. Roth, Arthr. Rheum., 12, 328 (1969).
70. H. Yamamoto, C. Saito, T. Okamoto, H. Awata, T. Inukai, A. Hirohashi, N. Yukawa, Arzneim.-Forsch., 19, 981 (1969).
71. Merck, Jap. Pat. 7698/69, published 4-11-69.
72. Sumitomo, Neth. Pat. 68,13867, published 4-1-69.
73. C. Colombini, G. Deganello, A. Benakis, M. Strolin-Benedetti, Helv. Chim. Acta, 52, 312 (1969).
74. B. Glasson, A. Benakis, M. Strolin-Benedetti, Biochem. Pharmacol., 18, 633 (1969).
75. J. R. Boissier, C. Frossard, P. Fabiani, Ann. Pharm. Fr., 26, 707 (1968)
76. J. Gougeon, J. Moreau-Hottin, S. Bajolet, J. C. Etienne, Therapie, 23, 951 (1968); G. Vignon, L. M. Patricot, J. P. Grandjean, M. Dechavanne, Rev. Lyon. Med., 17, 573 (1968).
77. R. Z. Gussin, J. R. Cummings, E. H. Stokey, M. A. Ronsberg, J. Pharmacol. Exptl. Therap., 167, 194 (1969).
78. K. Sota, K. Noda, H. Maruyama, E. Fujihara, M. Nakazawa, Yakugaku Zasshi, 89, 1392 (1969).
79. Council on Drugs, J. Am. Med. Assoc., 209, 2044 (1969).
80. Merck, Belg. Pat. 719414, published 2.13.69
Belg. Pat. 719416, published 2.13.69
Belg. Pat. 719417, published 2.13.69
Belg. Pat. 719418, published 2.13.69
81. S. Senda, H. Izumi, H. Fujimara, Yakugaku Zasshi, 89, 260 (1969).
82. S. Senda, H. Izumi, T. Tanimoto, H. Fujimura, Yakugaku Zasshi, 89, 254 (1969); S. Senda, H. Izumi, ibid., 89, 266 (1969); S. Senda, H. Izumi, K. Hirota, ibid., 89, 272 (1969).
83. S. B. Kadin, E. H. Wiseman, Nature, 222, 275 (1969).
84. I. K. Harrington, J. E. Robertson, D. C. Kram, R.R. Hamilton, K. T. McGurran, R. J. Trancik, K. F. Swingler, G. G. I. Moore, J. F. Gerster, J. Med. Chem., 13, 137 (1970).
85. U. Jahn, R. W. Adrian, Arzneim.-Forsch., 19, 36 (1969); W. Beckschäfer, Arzneim.-Forsch., 19, 52 (1969); Council on Drugs, J. Am. Med. Assoc., 208, 1400 (1969).
86. J. L. De Usobiaga, Rev. Exp. Reum., 12, 222 (1968).
87. J. Marques, V. Muriach, P. Barcelo, Rev. Exp. Reum., 12 229 (1968).
88. K. Trnavsky, O. Nemececk, 12th Intl. Rheum. Congress (Prague), 1969,

Abstr. No. 304; V. Cepelak, J. Lavicka, M. Belesova, H. Cepelakova, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 303; F. Kuthan, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 305.
89. H. Kopera, Excerpta Med. Int. Congr. Ser., 163, 100 (1968).
90. W. C. Dick, G. M. Cunningham, G. Nuki, M. K. Jasani, K. Whaley, Ann. Rheum. Dis., 28, 187 (1969); Council on Drugs, J. Am. Med. Assoc., 210, 1453 (1969).
91. C. Casagrande, A. Invernizzi, R. Ferrini and C. Miragoli, Farmaco Ed. Sci., 23, 1149 (1968); A. Boucherle, G. Carraz, M. I. Hicter, M. Boitard, J. P. Mazet and S. Lebreton, Chim. Therap., 3, 256 (1968).
92. D. A. Gerber, Arthr. Rheum., 12, 295 (1969); D. A. Gerber, L. Tannenbaum, Arthr. Rheum., 12, 296 (1969).
93. K. Miehlke, I. Kohlhardt, B. Wirth, Z. Rheumaforsch., 27, 445 (1968); P. Thular, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 604.
94. I. A. Jaffe, R. W. Smith, Arthr. Rheum., 11, 585 (1968).
95. G. Vetter, Munch. Med. Wochschr., 111, 1499 (1969).
96. Council on Drugs, J. Am. Med. Assoc., 208, 1399 (1969).
97. Y. Hiramatsu, Nippon Zasshi, 64, 608, 617 (1968); S. Nakamura, Y. Marumoto, H. Yamaki, T. Nishimura, N. Tanaka, M. Hamada, M. Ishizuka, T. Takeuchi, H. Umezawa, Chem. Pharm. Bull. 17, 714 (1969); S. Nakamura, Y. Marumoto, H. Miyata, I. Tsukada, N. Tanaka, M. Ishizuka, H. Umezawa, Chem. Pharm. Bull., 17, 2044 (1969).
98. T. R. Maxson, E. L. Compton, Ann. Allergy, 27, 54 (1969).
99. M. B. Gupta, T. N. Bhalla, G. P. Gupta, C. R. Mitra, K. P. Bhargava, Eur. J. Pharmacol., 6, 56 (1969); V. Yakovlev, A. von Schlichtegroll, Arzneim.-Forsch., 19, 615 (1969).
100. R. B. Filderman, B. A. Kovács, Brit. J. Pharmacol., 37, 748 (1969); R. B. Filderman, B. A. Kovács, Fed. Proc., 28, 357 (1969).
101. F. S. Sadritdinov, Farmakol. Toksikol., 32, 598 (1969); C. A., 72, 1988b (1969).
102. G. Wulff, R. Tschesche, Tetrahedron, 25, 415 (1969).
103. G. Mussgnug, Arzneim.-Forsch., 19, 1588 (1969); G. V. Obolentseva, Y. I. Khadzhai, Farmakol. Toksikol., 32, 174 (1969).
104. L. Kovács, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 471.
105. I. Yamagami, Y. Suzuki, M. Ito, H. Fujimara, Bitamin 39, 244 (1969); C. A., 71, 1908e (1969).
106. M. Rocha, E. Silva, R. Q. Cavalcanti, M. L. Reis, Biochem. Pharmacol., 18, 1285 (1969).
107. C. A. Youngdale, E. M. Glenn, D. Lednicer, J. Smuszkovicz, J. Med. Chem., 12, 948 (1969); H. P. S. Chawla, K. P. Grover, N. Anand, V. P. Kamboj, A. B. Kar, J. Med. Chem. 13, 54 (1970).
108. O. Kogstad, I. Rossel, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 843.
109. G. D. Kersley, Ann. Rheum. Dis., 29, 89 (1970).
110. F. Delbarre, C. Auscher, A. de Gery, H. Brouilhet, J. L. Olivier, Presse Med., 76, 2329 (1968).
111. I. Bruckner, O. Stroescu, V. Stroescu, M. Gheorghiu, M. Barnas, Arthritis and Rheumatic Diseases Abstr., 5, 417 (1969); C. J. Smyth, 12th Intl. Rheum. Congress (Prague), 1969, Abstr. No. 601.
112. P. Y. Patterson, D. G. Drobish, Science, 165, 191 (1969).
113. M. Mason, H. L. F. Currey, G. C. Barnes, J. F. Dunne, B. L. Hazleman,

I. D. Strickland, Brit. Med. J., 1, 420 (1969).
114. W. H. Dodson, J. C. Bennett, J. Clin. Pharmacol., J. New Drugs, 9, 251 (1969).
115. G. Klein, R. Kalb, Z. Rheumaforsch., 28, 1 (1969).
116. I. Bruckner, O. Stroescu, V. Stroescu, M. Gheorghiu, St. Suteanu, L. Ghelerter, P. Grozea, M. Barnas, Med. Interna., 21,583 (1969).
117. J. Gieldanowski, A. Pelczarska, J. Patowski, Z. Michalski, B. Szaga, Arch. Immunol. Ther. Exp. 17, 380 (1969).
118. J. L. Turk, D. A. Willoughby, J. E. Stevens, Immunology, 14, 683 (1968).
119. R. J. Perper, E. M. Glenn, R. E. Monovich, Nature, 223, 86 (1969).
120. G. Regnier, R. Canevari, J. C. LeDouarec, Z. Holstorp, Chim. Therap., 4, 174 (1969); G. Regnier, R. Canevari, J. C. LeDouarec, M. Laubie, J. Duhault, Chim. Therap., 4, 185 (1969); Council on Drugs, J. Am. Med. Assoc., 210, 714 (1969); A. Sabbah, Pharmacologist, 11, 266 (1969).
121. C. J. Paget, K. Kisner, R. L. Stone, D. C. DeLong, J. Med. Chem., 12, 1010, 1016, 1098 (1969).
122. J. Krapcho, R. C. Millonig, C. F. Turk, B. J. Amrein, J. Med. Chem., 12, 164 (1969); R. C. Millonig, J. Krapcho, 158th Meeting A. C. S., New York, Sept. 1969, Med. Chem. Abstr. No. 34.
123. E. M. Glenn, Proc. Soc. Exptl. Biol. Med.,129, 860 (1968).
124. G. D. Gray, M. M. Mickelson, J. A. Crim, Biochem. Pharmacol., 18, 2163 (1969); M. E. Greig, A. J. Gibbons, Arch. Intern. Pharmacodyn., 182, 364 (1969).
125. L. Szanto, F. Gorgengi, M. Benczur, A. Reviczky, B. Banyai, G. Lorincz, B. Gomor, Orv. Hetilep, 110, 181 (1969).

Chapter 21. Agents Affecting Thrombosis

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Introduction

This review is concerned with synthetic compounds that can affect thrombosis. The problem of thrombosis is an ever-growing one because of the increasing stress of civilization and the rising number of older people in the population. Several therapeutic approaches have been taken and this review will cover those synthetic compounds that can either prevent or interfere with the formation of a thrombus or can dissolve it after it has been formed. Because of the large number of papers published on this topic, the review will give only the highlights of work reported in 1969. For the purposes of this discussion, thrombolysis will be used interchangeably with fibrinolysis.

Fibrinolysis

This approach is based on the fact that fibrin forms the frame-work or structural support of a clot. Dissolution of the fibrin should result in lysis of the clot with restoration of blood flow. The significance of fibrinolysis[1], the potential of thrombolysis in treatment of thrombotic coronary occlusion[2] and fibrinolytic mechanisms[3,4,5] have been reviewed.

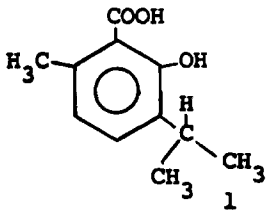
Streptokinase (SK) a bacterial protein, capable of activating the fibrinolytic system has been used clinically as a thrombolytic agent, but side effects of hypersensitivity and pyrogenicity have inhibited its development. It has been recently reported that it can cause the lysis of arterial clots that are several months old[6]. This finding does not accord with the experience of many other investigators that most lytic agents are ineffective if the clot is older than 5 days. SK has also been found to have an anti-histamine effect[7], to have a beneficial effect on healing of sutured arterial wounds in dogs[8] and to be able to directly activate human plasminogen to plasmin[9] (the actual lytic agent).

Human urokinase, a protein able to activate the fibrinolytic system, has been used therapeutically, but its high cost and inadequate supply have hampered its clinical development. It has been reported that it removes all but trace amounts of fibrin in kidney lesions produced in experimental glomerulonephritis and prevents the appearance of nephritic symptoms[10]. Other high molecular weight fibrinolytic substances are certain dextran fractions[11], brinase, an enzyme derived from Aspergillus oryzae[12], ateroid, a naturally occuring mucopolysaccharide complex[13] and erythrokinase (EK) a protein isolated from red blood cells that activates human and bovine plasminogen in a manner similar to urokinase but which has different physical properties[14].

Another approach to fibrinolysis has been the development of synthetic compounds capable of inducing increased fibrinolytic activity. Such compounds should be free of the side effects associated with protein or polysaccharide fibrinolytic agents (pyrogenicity and anaphylaxis) and should be much more inexpensive to produce.

Von Kaulla has systematically investigated many structural types[4,15] for activity and additionally has undertaken structure-activity studies in families that have shown activity. Among these are the phenylbutazones and certain members of the following family of acids: anthranilic, benzoic, indolecarboxylic, iminoproprionic, maleamic, naphthoic, phenylacetic,

phthalamic, resorcylic, salicylic and thiophene-3-carboxylic. Among the most active substances found were 5-(2'-chlorobenzyloxy)salicylic acid, O-thymotic acid 1, p-iodobenzoic acid, N-(3-trifluoromethyl-6-chlorophenyl)-anthranilic acid, and ketophenylbutazone.



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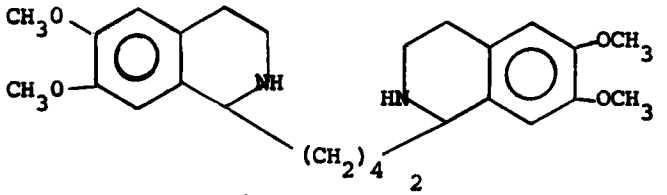
However, the activity tests used were all of the in vitro type with no results reported for in vivo systems. The same group has found that these compounds act[16] by inhibiting the naturally occurring plasma inhibitors of fibrinolysis thus permitting the fibrinolytic activity normally present in plasma to evince itself strongly. Hansch[4] has developed a physicochemical approach to studying structure-activity relationships of the above classes of fibrinolytics.

Anti-inflammatory compounds such as phenylbutazone, indomethacin, mefenamic acid and flufenamic acid have been reported by Gryglewski to have fibrinolytic activity[4]. Structure-activity studies with fenamates[4] have shown that two aryl rings bridged by a short chain and an electrophilic center represented by carboxylate or enolate ions are usually required for activity. Activity of these anti-inflammatory compounds appears to be due to inhibition of natural fibrinolytic inhibitors. Results in in vivo systems have not been reported. Among the structures reported by Desnoyers[4] to have lytic activity are 5-(2'-chlorobenzyloxy)salicylic acid and 2-hydroxy-5-(2'-methylthiazol-4'-yl)benzoic acid. He found these compounds to be active in vivo as well as in vitro.

Schor has published[4] on the in vitro fibrinolytic activity of bisobrin (EN-1661), a bis tetrahydroisoquinoline 2. This compound has been found by Moser to be active in the lysis of in vivo clots in the dog[4] and Ambrus has reported[4] on the promising clinical use of bisobrin.



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Fearnley has discussed[4] the pharmacological enhancement of fibrinolysis including the lytic effect of epinephrine, nicotinic acid, ACTH and insulin. The rebound increase of fibrinolytic activity at low blood-glucose levels in diabetics who had received an injection of insulin suggested that sulfonylurea compounds might also increase fibrinolytic activity. It was found that tolbutamide and chlorpropamide induced activity as did biguanides such as phenformin and metformin[4]. Phenformin[17] alone and in combination with ethylestrenol[18] has been used to increase fibrinolytic activity in man.

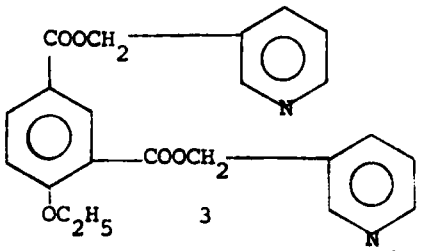
Several years ago, parenteral nicotinic acid was found to induce an intense but short-lived response in man[19]. Repeated injections led to the rapid development of tachyphlaxis which, combined with side effects, made the compound useless clinically. Newer derivatives and homologs of nicotinic

acid have been made and tested in man[20] including 3-pyridineacetic acid, nicotinic acid N-oxide, betaine nicotinate, nicofuranose and xanthinol nicotinate.[21] Betaine nicotinate and xanthinol nicotinate appeared to be the best compounds in the group, but more information is needed concerning their potency (by other analytical techniques) side effects and induction of tolerance. Comparable *in vivo* investigations were carried out in animals with similar results.[22] Included in these latter studies was nialamide, which was able to induce lytic activity as determined by increases in plasma plasmin. Isonicotinamide and isonicotinic acid were also found to induce *in vivo* increases in the fibrinolytic level in rabbits, whereas nicotinamide, picolinic acid and isoniazid had no effect on activity.[23] Long term administration of the isonicotinamide and isonicotinic acid led to the development of tachyphylaxis.

Streptomycin has been reported to induce a transient increase in lytic activity but kanamycin, monomycin and neomycin consistently inhibited fibrinolytic activity for 5-6 hours after injection.[24]

3-(ω-aminoalkylcarbamoyl)-4-hydroxy-1-thiocoumarin has also been reported to have fibrinolytic as well as choleretic activity but more experimental details must be given for proper assessment of this compound.[25]

A series of 4 hydroxyisophthalic acids was investigated for fibrinolytic and anticoagulant activity by *in vitro* assays.[26] Thromboelastography showed the most active compound to be the 4 ethoxy analog 3.



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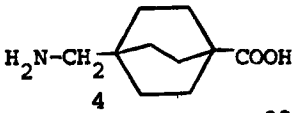
It had previously been reported that sodium gluconate possessed fibrinolytic activity[27] but more recent work has shown this compound to be ineffective in dissolving thrombi in cat arteries.[28]

The development of anti-fibrinolytic compounds to control hemorrhage due to excessive fibrinolytic activity has assumed more importance because of the increasing use of fibrinolytic therapy. A side effect of therapy with the protein activators SK and UK is destruction of plasma fibrinogen which causes hemorrhage. The subject of fibrinolysis inhibition has been reviewed by Baumgarten[4] and Maxwell.[4]

ε-aminocaproic (EACA) one of the earliest anti-fibrinolytic agents used therapeutically must be given in large doses because of its rapid elimination and low potency. Structural requirements for activity in this series have been reported.[29] More active compounds have been prepared namely 4-(aminomethyl)benzoic acid and trans-(aminomethyl)cyclohexanecarboxylic acid (t-AMCHA) which is 10 times more active than EACA. The activities of these inhibitors and trasylol a protein inhibitor isolated from pancreas have been compared in several inhibitor systems.[4,30,31] A new compound, 4-aminomethylbicyclo [2,2,2)-octane-1-carboxylic acid 4 has been shown to be about 60 times more potent than EACA[30,31] on a weight basis.



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Certain ω-guanidino fatty acid esters[32] and 2-thionotetrahydro-1,3,5-thiadiazine[33] are reported to have anti-fibrinolytic activity.

Pronethalol, the β-receptor blocker reduced, in humans, the release of plasminogen activator into plasma that normally occurs following an infusion of adrenaline.[34,35]

Anticoagulants

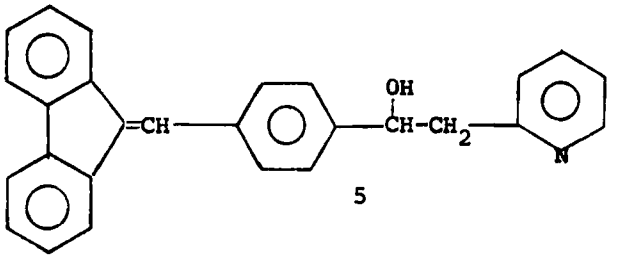
Compounds with this activity have been used therapeutically to prevent the formation of thrombi. It is a prophylactic approach, in contradistinction to the use of fibrinolytic agents for the treatment of already formed clots. Recent developments in the therapeutic use of anticoagulants have been reviewed[36-38] as has the blood coagulation system itself.[39-41] A variety of drugs may potentiate or interfere significantly with the activity of the coumarin and indandione anticoagulants and this has also been reviewed.[42-44]

The major metabolites of warfarin found in rat urine after ingestion of 4-C^{14} labelled compound are 7-hydroxywarfarin, 4-hydroxywarfarin, 6-hydroxywarfarin, 8-hydroxywarfarin and a glucuronide of 7-hydroxywarfarin.[45] Several thin-layer chromatographic systems for the identification of coumarin derivatives have been described[46] as has the comparative pharmacokinetics of bishydroxycoumarin and warfarin elimination in the rat, dog, rhesus monkey and man.[47-49] The plasma half-life increases in the order: rat> monkey> dog> man ($t_{1/2}$ at 3 mg./kg. is 37 hours). From a study of NMR and IR data intramolecularly hydrogen bonded structures have been proposed for dicoumarols, warfarin and dimethones and possible relationships between such hydrogen-bonded structures and biological activity were discussed.[50]

3,3'-(Bromobenzylidene)bis[4-hydroxycoumarin] has antivitamin K activity but is 10-20 times less potent than 3,3'(chlorobenzylidene)bis [4-hydroxy-coumarin].[51]

A newly discovered therapeutic synergism provided by the combined use of 5-fluorouracil (FU) and warfarin in a large series of patients with advanced cancer of the gastrointestinal tract has tentatively been related to selective disturbance in energy generating systems within the neoplastic cell. Analogous metabolic effects have been found in murine glioblastoma.[52]

Certain alpha-fluorenyl and alpha-fluorenylidene-tolyl derivatives of heterocyclic alcohols and ketones such as α-(α-fluoren-9-ylidene-p-tolyl)-2-pyridine ethanol 5 possess anticoagulant activity but the milligram potency is less than that of the more active coumarin derivatives.



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Polymers of ethylene maleic acid (EMA) and its divinyl ether congener (Pyran) have anticoagulant effects in fibrinogen clotting by thrombin. Measurements of clotting time, clot opacity, yield and structure indicate that different effects are produced at distinctly different and broad concentration ranges of polymer. Fibrinogen clotting is exquisitely sensitive to EMA over a low range, about 4 times more than to heparin.[54]

Sodium cephalothin (Kelfin) or cephaloridine in concentrations greater than 11 mg. per ml. of blood inhibit the clotting mechanism *in vitro*. This is thought to be due to inhibition of Factor V.[55]

Structure-activity relationship has been investigated in 1,3-indandione derivatives using the rabbit as the experimental animal. Derivatives with ethyl and tert-butyl radicals in the side chain had the highest activity.[56]

2-Phenylbenzo [b] thiophen-3-(2H)-one 1,1 dioxide was shown to exhibit both anticoagulant and anti-inflammatory activity. Various analogs were prepared and separation of the anti-inflammatory activity from the anti-coagulant effect was achieved.[57]

Anti-Platelet Aggregation

One of the primary events in the formation of a thrombus is the aggregation of platelets to form a layer or plug attached to newly exposed collagen fibers in the connective tissue beneath the damaged endothelium. The platelets subsequently release adenosine diphosphate (ADP) which results in the cohesion of additional platelets thus forming the nucleus around which fibrin is formed (by activation of the intrinsic coagulation system). Entrapment of red and white blood cells follows and a full-blown thrombus results.

The anti-platelet aggregation approach to therapy attempts to prevent the formation of the platelet nidus and is thus a prophylactic type of therapy.

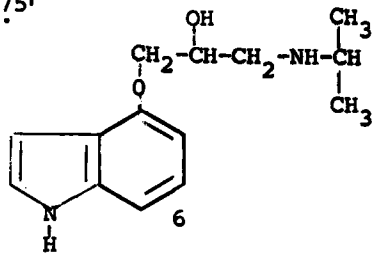
Platelet function,[58-60] platelets in myocordial infarction,[61] platelets in hemostasis,[62,63] platelet adherence to collagen[64] and inhibitors of platelet aggregation[65,66] have been reviewed. The effect of serotonin as an aggregator of platelets has been studied.[67] Procedures have been developed for measuring platelet production and turnover in the circulation and a physiological classification of platelet disorders in humans has been derived.[68]

Certain prostaglandins are among the most potent anti-platelet aggregation compounds known. Prostaglandin E_1 (PGE_1) inhibits ADP-induced platelet aggregation in citrated platelet-rich rat plasma, whereas prostaglandin E_2 stimulates it. Structure-activity studies with a series of prostaglandins showed that the C=0 group at the ring and in the carboxyl group, as well as the distance between them, are essential, as is the hydroxyl group at C-15.[69] PGE_1 inhibited platelet aggregation in rats given infusions of the compound at 1.8 mg./kg. per day for 30 days.[70] PGE_1 can be infused safely into man in small doses and this was found to decrease platelet adhesiveness.[71] However, the prostaglandins must be infused intravenously which makes their use as prophylactic agents difficult.

PGE_1 increases the level of cyclic AMP synthesized from ATP in human blood platelets[72,73] while epinephrine causes a decrease.[73] *In vitro* aggregation of blood platelets by catecholamines appears to be mediated through α-adrenergic receptors, as evidenced by the relative effectiveness of the catecholamines, adrenaline> noradrenaline> isoprenaline, and the complete inhibition of this classical α-receptor blockers such as phentolamine. The β-adrenergic blocking agent propranolol has no effect on the action of

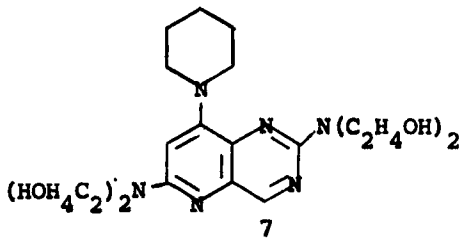
adrenaline.[73] However, stimulation of β-receptors by agents such as isoprenaline, in the presence of phentolamine, causes clumped platelets to disaggregate and this effect is mediated through increased formation of cyclic AMP.[74]

Propranolol can inhibit in vitro ADP induced platelet aggregation but the Sandoz compound LB-46 (6), a β-receptor blocking agent, had a 5 fold greater effect on the platelets.[75]



6

It has been known for several years that dipyramidole (Persantin) inhibits platelet aggregation. This compound and two new derivatives, RA 233 (7) and RA 433 (8) were compared as regards their inhibitory action on ADP and noradrenaline-induced platelet aggregation and adhesiveness in vitro. Adhesiveness and aggregation were markedly inhibited by 7 and 8 at 5-50 μg/ml while dipyridamole at 50-100 μg/ml. showed only a weak and irregular effect.[76]



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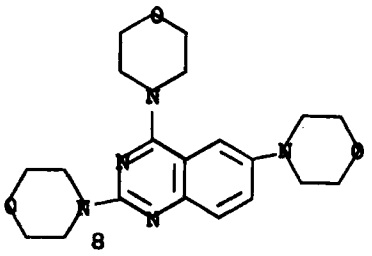

8

A single dose of 100 milligrams of dipyridamole given orally was reported to result in a significant decrease in irreversible platelet aggregation when the bloods of patients were tested in vitro.[77] In another clinical test, however, dipyridamole, in a dosage of 600 mg. daily for 2 weeks, given to 17 patients in whom the normal level of platelet adhesiveness was elevated, failed to influence this parameter of platelet function.[78]

Dipyridamole in combination with heparin or phenindione has been reported to prevent not only thrombosis in vessels during acute rejection but also the progressive narrowing of vessels characteristic of "chronic" rejection in cadaveric renal allografts. This supports the view that the intimal sclerosis which causes narrowing in the vessels of renal allografts results from deposition of mural thrombi.[79]

Platelet thrombi (aggregated platelets) in mesenteric arteries of the rat were produced by passing current through electrodes placed in the vessel walls. Dipyridamole induced a marked shortening in the duration of complete occlusion of the spastic vessel. Whether its effectiveness was due to its vasodilator action or its ability to inhibit platelet aggregation is not certain.[80]

Non-steroidal anti-inflammatory agents have been investigated for their effect on platelets. Some of these agents inhibit the release of LDH and

ACPase from thrombin-aggregated platelets in vitro but there does not appear to be a direct relationship between these effects on platelets and anti-inflammatory action.[81]

In in vitro experiments benzyl alcohol and phenol showed inhibitory effects on ADP-induced platelet aggregation and platelet adhesiveness. Benzyl alcohol is commonly used as a preservative in solutions for parenteral administration. In contrast, ethanol and isopropanol show no inhibitory effects.[82]

2-Methylthioadenosine-5'-phosphate inhibits the ADP-induced aggregation of human platelets in vitro. Its potency was approximately that of AMP but about one-eighth that of adenosine.[83] 2-Chloradenosine 5'-phosphate inhibited the ADP aggregation of sheep platelets with a potency equivalent to AMP.[84] The effect of other adenine compounds on platelets and their metabolic interconversions have been reported.[85]

Derivatives of 1,3-bis-[6,7-dimethoxy-3,4-dihydroisoquinoline-(1)]-2-arylpropane reduce platelet adhesiveness, aggregation, retraction and liberation of biogenic amines from cells in which these compounds have accumulated.[86] Derivatives of 3-alkyl-3-carbalkoxy-6,7-dimethoxy-3,4 dihydro-isoquinolines also have the same effect on platelets.[87]

Platelets treated with 1-p-chlorostyryl-3-methyl-3-carbomethoxy-6,7-dimethoxy-3,4 dihydroisoquinoline (SSDHI) lose their ability to spread, adhere, aggregate and retract. They cannot undergo the morphological and biochemical changes of viscous metamorphosis produced by thrombin.[88]

Elatericin A, a carcinostat isolated from Ecballium elaterium (cucurbitaceae) caused spicule formation on human platelets and inhibited spreading on glass surfaces, viscous metamorphosis and clot retraction. A normal ADP-release is obtained upon addition of thrombin to elatericin A treated platelets. Platelets treated with the substance up to a limiting concentration of 30 μg/ml. regain their clot retracting activity if washed and reincubated in normal plasma.[89]

REFERENCES

1. A.A. Sharp, Proc. Roy. Soc. B. 173, 311 (1969).
2. J.R. Hampton, Suppl. IV to Circ. 39 and 40, (Nov. 1969).
3. G.P. McNicol, Proc. Roy. Soc. B. 173, 285 (1969).
4. J.M. Schor, Ed., Chemical Control of Fibrinolysis-Thrombolysis, John Wiley, New York, 1970.
5. V. Hiemeyer, Ed., Therapeutische and Experimentelle Fibrinolyse, F.K. Schatlauer Verlag, Stuttgart, 1969.
6. H. Poliwoda, K. Alexander, V. Buhl, D. Holsten and H.H. Wagner, New England J. Med., 280, 689 (1969).
7. V.G. Fisher, Arzeim-Forsch., 19, 2017 (1969).
8. J. Safar, J. Gajewski, D.P. Shedd and E.F. Schueller, J. Surg. Res., 9, 649 (1969).
9. L. Summaria, B. Hsieh, W.R. Groskopf and K.C. Robbins, Proc. Soc. Exp. Biol. Med., 130, 737 (1969).
10. L. Humair, H.C. Kwaan and E.V. Potter, J. Lab. Clin. Med. 74, 72 (1969).
11. W.J. Cunliffe and I.S. Menon, Br. J. Derm., 81, 220 (1969).
12. C.L. Huang, E.T. O'Brien and R.D. Thornes, I.J. Med. Sc., 2, 379 (1969).
13. G. Prino and M. Mantovani, Europ. J. Pharmacol., 6, 190 (1969).
14. M. Semar, L. Skoza, and A.J. Johnson, J. Clin. Invest. 48, 1777 (1969).

15. K.N. von Kaulla, Thromb. Diath. Haemorrh Suppl. 32, 64 (1969).
16. N. Aoki and K.N. von Kaulla, Thromb. Diath. Haemorrh, 22, 251 (1969).
17. E. Fiaschi, T. Barbui, G. Previato, V. Di Luzio, G.F. Guarnieri and S. Todesco, Arzeim-Forsch., 19, 638 (1969).
18. G.R. Fearnley, R. Chakrabarti and J.F. Evans, The Lancet, 1, 910 (1969).
19. M. Weiner, Am. J. Med. Sci., 246, 294 (1963).
20. P. de Nicola, G. Frandoli and A. Gibelli, Proceed. of 7th Int. Cong. Gerontol, Vienna, p. 441 (1966).
21. P. de Nicola, G. Cultrea and G. Manai, Il. Farmaco, Ed. Sc., 23, 994 (1968).
22. P. de Nicola, Settimana Medica, 56, 1057 (1968).
23. K.M. Lakin, A.P. Gavrilov and A.I. Maiskii, Teor, Klin. Vop. Probl. Svertyvaniya, Krovi p. 108-11 (1968).
24. A.Y. Ivelva, Farmakol. Toksikol. (Moscow), 32, 321 (1969).
25. E. Boschetti, D. Molho. L. Fontaine, Fr. 1,521,366 to LIPHA, Apr. 19, 1968.
26. G. Orzalesi, R. Selleri and O. Caldini, Il. Farmaco, Ed. Sci., 24, 522 (1969).
27. P.H. Kopper, Nature (Lond.), 211, 43 (1966).
28. H. Rasche, V. Hiemeyer and K. Schniep, Thromb. Diath. Haemorrh., 20, 555 (1968).
29. A.J. Johnson, L. Skoza and A.O. Tse, Int. Symp. on Therapeutische und Exp. Fibrinolyse, 29, 105 (1967).
30. M. Matsuoka, N. Sakuragawa and Y. Yoneyama, Acta, Med. et. Biolog., 16, 105 (1969).
31. W. Baumgarten, L.I. Priester, D.W. Stiller, A.E.W. Duncan, J.L. Ciminera and L.J. Loeffler, Thromb. Diath. Haemorrh; 22, 263 (1969).
32. S. Fuju, W. Ger. 1,804,999, May 22, 1969.
33. G. Wuerbach and H.P. Kloecking, E. Ger., 63,506, Sept. 5, 1968.
34. J.D. Cash and D.G. Woodfield, The Lancet I, 54 (1969).
35. G.I.C. Ingram and R.V. Jones, The Lancet I, 310 (1969).
36. I.S. Wright, Ann. Int. Med, 71, 823 (1969).
37. J.R. Hampton and R. Gorlin, Am. J. Med. Sciences, 258, 1 (1969).
38. J.A. Udall, G.P., 40, 117 (1969).
39. W.A. Seegers, Ann. Rev. Physiol. 31, 1025 (1969).
40. R.G. Macfarlane, Proc. Roy. Soc. B. 173, 261 (1969).
41. M.P. Esnouf, Proc. Roy. Soc. B., 173, 269 (1969).
42. M. Formiller and M.S. Cohon, Am. J. Hosp. Pharm., 26, 574 (1969).
43. Am. J. Pharm., 141, 116 (1969).
44. M.G. Mac Donald, D.S. Robinson, D. Sylvester and J.J. Jaffe, Clin. Pharmacol and Therapeut., 10, 80 (1969).
45. W.M. Barker, M.A. Hermodson and K.P. Link, J. Pharm. Exp. Therapeutics, 171, 307 (1970).
46. von G. Kamm, Arzeim-Forsch., 10, 1336 (1968).
47. R. Nagashima and G. Levy, J. Pharm. Sci, 58, 845 (1969).
48. R. Nagashima, R.A. O'Reilly and G. Levy, Clin. Pharmacol and Therapeutics, 10, 22 (1969).
49. G. Levy and R. Nagashima, J. Pharm. Sci., 58, 1001 (1969).
50. D.W. Hutchinson and J.A. Tomlinson, Tetrahedron, 25, 2531 (1969).
51. M. Eckstein, J. Sulko and K. Jakubowski, Diss. Pharm. Pharmacol, 21, 147 (1969).
52. W.M. Kirsch, J.W. Leitner, D. Schulz, J.V. Burkirk and J. Sheflin, Surgical Forum, 20, 444 (1969).

53. E.M. Roberts, G.P. Claxton and F.G. Fallon, U.S. 3,481,929, Dec. 2, 1969 to Richardson-Merrell.
54. Y. Shamash and B. Alexander, Biochem et Biophys. Acta, 194, 449 (1969).
55. G. Raccuglia and N.G. Waterman, Tech. Bull. of Registry of Med. Technol. 39, 245 (1969).
56. V.I. Zavrazhnov, L.P. Zalukaev, Y.E. Dushenko, L.I. Trukhacheva, L.G. Lokhmatova, I.K. Anokhina and I.A. Ryazonova, (USSR) Tr. Voronezh. Gos. Med. Inst. 61, 151 (1968).
57. J.G. Lombardino and E.H. Wiseman, J. Med. Chem., 13, 206 (1970).
58. A.J. Marcus, New Eng. J. Med., 280, 1213 (1969).
59. A.J. Marcus, New Eng. J. Med., 280, 1278 (1969).
60. A.J. Marcus, New Eng. J. Med., 280, 1330 (1969).
61. J.F. Mustard and M.A. Packham, Suppl 4 to Circ., 20 (1969).
62. D.P. Thomas, Exp. Biol. Med., 3, 129 (1968).
63. E.F. Luscher, Thromb. Diath. Haemorrh., Suppl. 35, 149 (1969).
64. J. Hugues, Exp. Biol. Med., 3, 145 (1968).
65. A.J. Hellem and H. Stormorken, in Recent Adv. in Blood Coag., L. Poller Ed., Little Brown, Boston, 1969, p. 69.
66. M.J.G. Harrison, Exp. Biol. Med., 3, 175 (1968).
67. H.R. Baumgartner and G.V.R. Born, J. Physiol., 201, 397 (1969).
68. L.A. Harker and C.A. Finch, J. Clin. Invest., 48, 963 (1969).
69. J. Kloeze, Biochem et Biophys. Acta, 187, 285 (1969).
70. N.C. Sekhar, J. Med. Chem., 13, 39 (1970).
71. R.S. Elkeles, J.R. Hampton, M.J.G. Harrison and J.R.A. Mitchell, The Lancet, II, 111 (1969).
72. R.L. Vigdahl, N.R. Marquis and P.A. Tavormina, Biochem, Biophys, Res. Comm., 37, 409 (1969).
73. G.A. Robison , A. Arnold and R.C. Hartmann, Pharmacol. Res. Comm., 1, 325 (1969).
74. Y.H. Abdulla, J. Atheroscler. Res., 9, 171 (1969).
75. H.W. Bucher and P. Stucki, Experientia, 25, 280 (1969).
76. R. Eliasson and S. Bygdeman, Scand. J. Clin. and Lab. Invest. 24, 145 (1969).
77. C.D.W. Morris, Arzeim-Forsch., 19, 1315 (1969).
78. E.E. Mayne, J.M. Bridges and J.A. Weaver, J. Atheroscler. Res., 9 335, (1969).
79. P. Kincaid-Smith, The Lancet, II, 920 (1969).
80. P. Didisheim, Thromb. Diath. Haemorrh., 20, 258 (1968).
81. M. Glenn and B.J. Bowman, Proc. Soc., Exp. Biol. Med., 130, 1327 (1969).
82. A.J. Zweifler and S.S. Sanbar, Thromb. Diath. Haemorrh., 21, 362 (1969).
83. F. Michal, M.H. Maguire and G. Gough,Nature, 222, 1073 (1969).
84. G. Gough, M.H. Maguire and F. Michal, J. Med. Chem. 12, 494 (1969).
85. M.A. Packham, N.G. Ardlie and J.F. Mustard, Am. J. Physiol., 217, 1009 (1969).
86. Belg. 718,393 (to VEB Arzneimittelwork, Dresden), Jan. 22, 1969
87. Belg. 718,394 (to VEB Arzneimittelwork, Dresden), Apr., 1969
88. F. Markwardt, W. Barthel, E. Glusa and A. Hoffmann, Thromb. Diath. Haemorrh., 21, 380 (1969).
89. H. Joshua, B. Shohat, I. Cohen and S. Gitter, Thromb. Diath. Haemorrh., 21, 346 (1969).

Section V - Topics in Biology

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Chapter 22: Drug Metabolism

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Introduction - This chapter will focus on data published in 1969 concerning the metabolic fate of various classes of therapeutic agents. Agents affecting the CNS, particularly psychoactive agents, appeared to be the most frequently studied compounds. Investigations describing only the absorption, excretion, and distribution of drugs will not be mentioned unless such studies were of particular significance. Structural formulas have been provided for new compounds or for those not readily available in The Merck Index. An attempt has been made to emphasize those biological and chemical aspects that influenced the biotransformation of a molecule, as well as those instances that resulted in the formation of pharmacologically active metabolites.

Psychotherapeutic agents - Perazine underwent a series of metabolic transformations similar to those previously reported for other phenothiazines.[1] Urine recovered from schizophrenic patients dosed with perazine contained N-oxide derivatives as the major nonhydroxylated metabolites, whereas this was not the case for chlorpromazine.[1,2] The author surmises that this finding can be explained by the presence in perazine of three nitrogen atoms and an N-oxide group that results in the formation of a hydrophilic molecule that is no longer reabsorbed in the kidney tubules. Nine metabolites isolated from the urine were the result of demethylation, sulfoxidation, N-oxidation, 3-hydroxylation, and conjugation, or a combination of these processes.[3] One of the metabolites was identified as the piperazine-2,5-dione derivative of perazine sulfoxide.[4] During treatment with perazine, patients exhibited a consistent increase in the demethylated products relative to the corresponding tertiary amines, a finding attributed to the possible induction of a liver microsomal demethylase.[3]

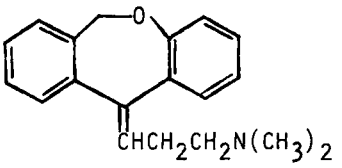
The metabolism of promazine, a compound useful in veterinary practice, was studied in horses.[5] Eleven percent of the dose was excreted in the urine. The major metabolite was the glucuronide conjugate of 3-hydroxypromazine, but at least eight other metabolites were detected.

At least 20 metabolites of imipramine, excreted in human urine, were identified by two-dimensional TLC.[6] These metabolites resulted from a combination of mono- or di-demethylation or dealkylation of the side chain and hydroxylation at the 2 or 10 position with subsequent conjugation. Imipramine N-oxide was also detected as well as a new, unidentified conjugating group.

Whole-body autoradiography of mice was employed as the technique to study the distribution of diazepam, chlordiazepoxide, and their metabolites.[7] The former compound was more highly localized in body fat than the latter; however, the rate of penetration of chlordiazepoxide into the brain was slower than that of diazepam. The major metabolite of diazepam was the N-demethylated compound. In vitro, in preparations of rat or mouse liver microsomes, the major metabolites of diazepam were N-methyloxazepam or N-demethyldiazepam, respectively.[8] Diazepam inhibited the conversion by mouse liver microsomes of N-methyloxazepam to oxazepam. Prazepam, the cyclopropyl derivative of the N-methyl group of diazepam, was metabolized by the dog in a manner similar to that for diazepam; the major urinary metabolite was oxazepam glucuronide.[9]

Doxepin (I) was metabolized by rats and dogs via demethylation, N-oxidation, hydroxylation, and glucuronide formation; uncharacterized polar metabolites were also detected in the urine of both species.[10]

Flupenthixol, a substituted thioxanthene, was metabolized by rats and dogs by sulfoxidation, partial dealkylation of the piperazine-containing side chain and O- and N-glucuronide formation.[11] Phenolic metabolites were not detected.



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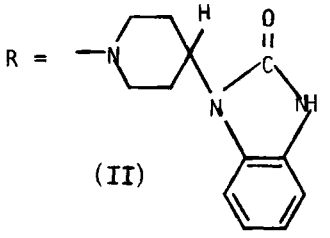

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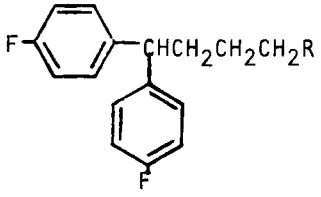
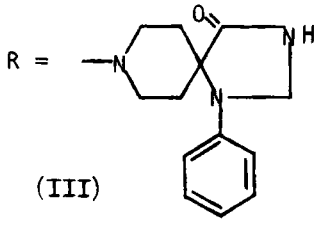




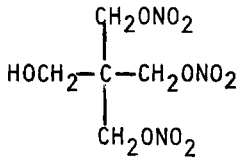
(III)

The biotransformation of pimozide (II) was studied employing either the ^{3}H- or ^{14}C-labeled compound.[12] When ^{3}H-pimozide was administered s.c. to rats, the radioactivity was excreted primarily in the feces as 4-bis(p-fluorophenyl) butyric acid and bis(p-fluorophenyl)acetic acid. When ^{14}C-pimozide was administered s.c. to rats, the main metabolite excreted in the urine was the dealkylation product, N-4-piperidyl-2-benzimidiazolinone.[12] Fluspirilene (III), a compound structurally related to pimozide, was ad-

ministered i.m. to rats.[13] When ^{3}H-fluspirilene was given, the same metabolites cited above for ^{3}H-pimozide were detected in the feces. When ^{14}C-fluspirilene was given, the major metabolite in the urine was 1-phenyl-1,3,8-triazaspiro[4,5]decan-2,4-dione; phenylurea and p-hydroxyphenylurea were detected in the feces.

Cardiovascular agents - The metabolism of quinidine, a naturally occurring alkaloid with antiarrhythmic properties, was studied in man as the gluconate salt.[14] Metabolites isolated from urine were found to be oxygenated on either the quinoline or quinuclidine portions of the molecule; uncharacterized polyoxygenated derivatives were also found. Commercially available supplies of quinidine consistently contained 10 to 30 percent of an impurity.

Glyceryl trinitrate was converted by rabbits to the mono-and 1,3-dinitrate derivatives, which were excreted in the urine.[15] The isomeric 1,2-glyceryl dinitrate was not detected as a metabolite of glyceryl trinitrate. After the administration of 1,2-glyceryl dinitrate, only glyceryl mononitrate (isomeric position unknown) was detected. The metabolism of pentaerythritol trinitrate (IV), an active metabolite of pentaerythritol tetranitrate, was studied in dogs.[16] The major metabolites in the urine were pentaerythritol mononitrate and pentaerythritol; only the latter compound was present in the feces. An oral dose of pentaerythritol was excreted unchanged by the dog.[16]

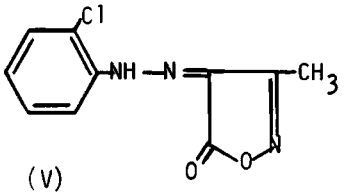


(IV)

Reserpine, tritiated in the 2- and 6-positions of the trimethoxybenzoyl ring, was administered orally to human subjects.[17] The major urinary metabolite was ^{3}H-trimethoxybenzoic acid, while most of the radioactivity in the feces, the predominant route of excretion, was unaltered reserpine.[17]

Chemotherapeutic agents - Isomeric mono- and di-methoxy-6-sulphanilamidopyrimidines were given orally to man and animals.[18] In man the 4-methoxy compound was much better acetylated than were the 2,4- and 4,5-dimethoxy compounds. The main metabolite of the 2,4- but not of the 2,5- or 4,5-dimethoxy compound, was the N^{2}-glucuronide. Glucuronide formation of the 2,4-dimethoxy compound occurred in the rhesus monkey but not in the rabbit and only to a small extent in the rat. In a similar study with sulphasomidine and sulphamethomidine, the former compound was excreted primarily unchanged in the urine of man and animals with some acetylation occurring, while the latter compound was primarily excreted as the N^{2}-glucuronide in the urine of man and the rhesus monkey, but not in the urine of the rat or rabbit.[19]

The metabolism of an arylazoisoxazolone and a chlorinated analogue, drazoxolon (V), was studied in rats and dogs.[20] The major metabolite of drazoxolon in both species was 2-(2-chloro-4-hydroxyphenylhydrazone)aceto-acetic acid. The rat excreted the sulfate ester and the O-glucuronide, but the dog excreted only the glucuronide conjugate. The biotransformation of the nonhalogenated compound, 3-methyl-4-(phenylhydrazono)isoxazol-5-one, by the rat was not different from that of drazoxolon.[20]



(V)

Hetacillin, the acetone derivative of ampicillin, was shown to be rapidly converted to the latter compound in the plasma of man, indicating that the clinical efficacy of hetacillin was a result of its conversion to ampicillin.[21] Griseofulvin was converted by dogs to the 6-demethylated compound and its glucuronide.[22]

Insecticides - The metabolism of DDT and its dichloro derivative, DDD, was studied in pigeons.[23] DDT was converted to either 1,1-di(p-chlorophenyl)-2,2-dichloroethylene (DDE) or DDD, while DDD was converted mainly to 1,1-di(p-chlorophenyl)-2-chloroethylene (DDMU) together with a trace of DDE. The authors propose that DDE may be the causative agent responsible for the large increase in liver weights observed in pigeons fed DDT but not in those fed DDD. Kelthane, the hydroxy derivative of DDT, was administered p.o. or i.p. to rats.[24] DDE, 4,4'-dichlorobenzophenone, and 4,4'-dichlorobenzhydrol were found as metabolites in tissues and excreta.

Dihydroaldrin was detoxified by houseflies and by housefly and pig liver microsomes primarily to a mixture of the 6-exo- and 6-endo-hydroxy derivatives.[25] Microsomes from pig liver and houseflies also convert aldrin, by epoxide formation, to the toxic product dieldrin. Based on studies with compounds that are known inhibitors of steroid epoxidation and hydroxylation, evidence is presented that a common enzyme system is responsible for the oxidative conversion of steroids and aldrin and dihydroaldrin.

Steroids - The metabolism of orally administered ethynylestradiol-6,7-^{3}H (EE) and its 6,7-^{3}H-3-cyclopentyl-1-^{14}C ether (EECPE) was studied in patients with externalized bile ducts.[26] EECPE was cleaved either to EE and cyclopentanol derivatives (urinary products) or to various conjugation products of compounds containing an intact cyclopentyl ether linkage (urinary and biliary products). In patients given EE, the unaltered compound was the main excretory product in the glucuronide and sulfate fractions of urine and bile. Other more polar metabolites were also excreted, two of them presumed to be 6α-hydroxy EE and 6α-hydroxy EECPE.

In another study, estrone-6,7-^{3}H-sulfate-^{35}S was administered i.v. to a woman with a bile fistula.[27] Of particular interest was the presence of the sulfo-N-acetylglucosaminide (double conjugate) of 15α-hydroxyesterone

and 15α-hydroxyestradiol. These doubly conjugated compounds were more concentrated in the bile than in the urine.

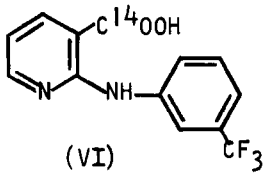
Metabolism of stereoisomers - The metabolism of orally administered (+)-, (-)-, and (±)-N-ethylamphetamine was studied in man.[28] When subjects excreted an acidic urine, the (+)-isomer was deethylated to the metabolite, amphetamine, more readily than was the (-)-isomer. By studying the effect of the nature of the N-alkyl substituent, the authors concluded that N-methyl or N-ethyl substitution produced relatively more N-dealkylation of the (+)- but not of the (-)-isomer.

A study of the pharmacological effects of the enantiomers of hexobarbital in rats revealed a stereoselective disposition.[29] The onset of hexobarbital-induced sleep was more rapid with the d- than with the l-enantiomer. Whereas either enantiomer of hexobarbital produced about equal sleeping times in male rats, d-hexobarbital was several times more potent than l-hexobarbital in producing sleep in females. In another study, the biotransformation of R(+)- and RS-pentobarbital was investigated in dogs that received the compounds i.v.[30] After the administration of R(+)-pentobarbital, the major urinary metabolites were 5-ethyl-5-[3'(R)-hydroxy-1'(R)-methylbutyl]- and 5-ethyl-5-[3'(S)-hydroxy-1'(R)-methylbutyl]barbituric acid. Similar products were obtained after dogs had been given RS-pentobarbital, one metabolite being derived from R(+)-pentobarbital and the other from S(-)-pentobarbital.

The metabolism of the camphor compounds (±)-norcamphor, (+)-camphor, (-)-camphor, (+)-epicamphor, (±)-camphorquinone, (±)-camphane-2,5-dione, and camphane, was studied in rabbits.[31] No pharmacological activity was evident after oral dosing with (±)-norcamphor, (±)-camphorquinone, or camphane. Biotransformation occurred by hydroxylation of a methylene group, reduction of an oxo group, and subsequent glucuronide conjugation. Hydroxylation of (+)- and (-)-camphor produced the 5- and 3-endo derivatives, the former predominating. These results are consistent with the more strained nature of the 5- versus the 3-position of camphor. Of the ketones investigated, all were reduced in vivo, but only (±)-norcamphor and (±)-camphorquinone were reduced in vitro by alcohol dehydrogenase, suggesting that some other enzymatic mechanism must be responsible for the reduction of the other ketones.

The hydroxylation of trans-stilbene, trans-α,β-diphenylethylene, was studied in rabbits and guinea pigs after i.m. administration.[32] The metabolites found in the urine of both species were 4-hydroxy-, 4,4'-dihydroxy-, 4-hydroxy-3-methoxy-, and 3-hydroxy-4-methoxy-stilbene and their unspecified conjugates. When rabbits were given 4-hydroxystilbene, the 4,4'-dihydroxy compound was found in the urine. The reduction product, 4,4'-dihydroxybibenzyl, was also found in rabbit urine.

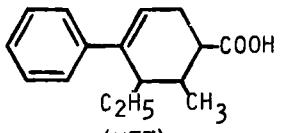
Metabolism of other drugs - The metabolism of niflumic acid (VI), an anti-inflammatory agent, was studied in the rat after i.p. administration.[33] The parent compound was completely converted to at least five urinary metabolites, one of which was identified as 2-aminonicotinic acid; about 5% of



(VI)

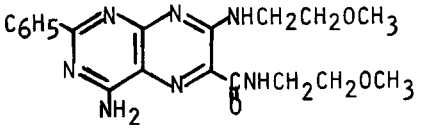
the dose appeared as $^{14}CO_2$. Glucuronide conjugation of the acidic moiety of niflumic acid was also observed. The antihistaminic, chlorpheniramine-^{3}H, was given orally to rats and dogs.[34] The urinary radioactivity that was extracted and identified consisted of a trace of unaltered chlorpheniramine and mono- and di-dealkylated products, the latter predominating. N-dealkylation was also the major metabolic pathway in man.[35]

The biotransformation of the nonsteroidal, postcoital oral contraceptive, 2-methyl-3-ethyl-4-phenyl-Δ^4-cyclohexene carboxylic acid (VII), was examined in monkeys and rabbits.[36] In both species, only a trace of unaltered drug was found in the urine. At least eight metabolites were separated from rabbit urine, one of which, as yet unidentified, had 1.5 to 2.0 times the estrogenic potency of the parent compound. The antifertility effect, in the rat of certain p-substituted, but not of unsubstituted compounds, was not blocked by prior treatment of the animals with SKF-525A. Thus, the authors suggest that when hydrogen alone is in the para position, further alteration of the molecule is necessary to achieve an antifertility effect.



(VII)

The metabolism of two diuretics was reported. One of them, a pteridine compound, 4-amino-N-(2-methoxyethyl)-7-(2-methoxyethylamino)-2-phenyl-6-pteridinecarboxamide (VIII), was studied, after oral dosing, in the rat, dog, and squirrel monkey.[37] All three species transformed the drug by successive O-demethylation of both aliphatic ether side chains to isomeric mono-demethylated metabolites and the major metabolite, a bis-demethylated product. Mono-demethylation yielded products that still retained some diuretic activity. The other diuretic amiloride hydrochloride, was given orally to man.[38] All of the radioactivity excreted was identified as unaltered amiloride.



(VIII)

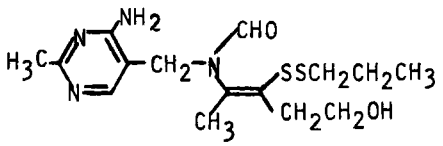
Metabolites of chlorphenesin, an antiallergic agent, were identified from rat and dog urine.[39] After oral dosing, in addition to unchanged drug, four products were identified. In the order of their abundance they were 3-p-chlorophenoxylactic acid, p-chlorophenoxyacetic acid, a conjugate of chlorophenol, and a conjugate of chlorphenesin. Chlorpropamide, an oral hypoglycemic, was shown to undergo metabolic transformation in man; two of the products were identified as p-chlorobenzenesulfonylurea and p-chlorobenzenesulfonamide.[40] Interestingly, two patients failed to excrete either

of the cited metabolites, reflecting, the authors believe, the rapid conversion of these metabolites or chlorpropamide to other products.

The metabolism of pentazocine, an analgesic, was studied in rat, mouse, and monkey liver homogenates and in the monkey *in vivo*.[41] In both cases, modifications occurred to the dimethylallyl side chain, yielding two isomeric alcohols and one of the resulting carboxylic acids. Monkey urine also contained unidentified conjugates and possibly unextracted metabolites. *In vitro*, the different species produced the isomeric alcohols in varying amounts. Man appears to metabolize pentazocine generally like the monkey.[41] The anticonvulsant, phenurone (phenacetylurea), was converted by rabbits,[42] but not by mice,[43] to 3-methoxy-4-hydroxyphenacetylurea. Other metabolites produced by the mouse and excreted in the urine included 4-hydroxyphenacetylurea, phenaceturic acid, phenylacetic acid, and the glucuronides of 4-hydroxyphenacetylurea and 4-hydroxyphenylacetic acid.[43] The authors propose that the formation of a dihydroxy precursor of 3-methoxy-4-hydroxyphenacetylurea occurs poorly in the mouse, if at all.

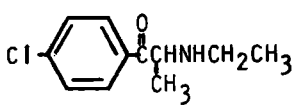
The metabolism of a variety of hydroxy- and methoxy-substituted cinnamic acids (derivatives of sinapic acid) was studied after oral administration to rats.[44] A series of urinary metabolites was excreted, resulting from p-dehydroxylation, demethylation, and reduction of the double bond of the cinnamic acid side chain to give 3-hydroxy-5-methoxy-phenylpropionic acid. Studies indicated that the 3,4,5- or 3,5-substituted cinnamic and phenylpropionic acids were not converted to the corresponding benzoic acids or their metabolites. Tremorine metabolism was studied in rats given the drug i.p.[45] Symmetric dioxotremorine was identified as a urinary metabolite that had no demonstrable biological activity in rats and mice. By contrast, oxotremorine, another metabolite of tremorine, produces tremor and salivation in rats and mice, and a pronounced hypothermia in mice.

Thiamine propyl disulfide-^{35}S, a thiamine substitute (IX), was studied in rabbits and man.[46] The propyl mercaptan moiety of the compound was excreted primarily in the urine as methylpropylsulfone and its 2- and 3-hydroxy derivatives. In addition, the rabbit was found to excrete methylsulfonylpropionic acid and inorganic sulfate.



(IX)

The anorectic agent, 4'-chloro-2-ethylaminopropiophenone (X), was the subject of another study in man.[47] The recognized metabolites were N-de-ethylated (4'-chloro-2-aminopropiophenone), reduced (1-[4-chlorophenyl]-1-hydroxy-2-ethylaminopropane) and reduced and N-de-ethylated (1-[4-chlorophenyl]-1-hydroxy-2-aminopropane). The first two of these metabolites were rapidly formed from the parent drug and excreted in the urine. By



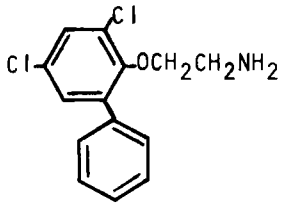
(X)

having the subjects produce an acidic urine, the authors were able to minimize tubular reabsorption of the amines, thereby increasing the total amount of excretion of the parent drug and its products. In this way, urinary excretion was employed as an indicator of the biological availability of different dosage forms and regimens.

The metabolism of probenecid (p-[di-N-propylsulfamyl] benzoic acid), a uricosuric agent, was investigated in rats with ligated renal pedicles.[48] After i.v. administration, 96% of the dose was excreted in eight hours in the bile. Four major metabolites, resulting from side chain hydroxylation followed either by glucuronide conjugation or further oxidation, were identified as p-(N-propyl,N-2-hydroxypropylsulfamoyl)benzoic acid, p-(propylsulfamoyl)benzoic acid, p-(N-propyl,N-3-hydroxypropylsulfamoyl)benzoic acid, and p-(N-propyl,N-2-carboxyethylsulfamoyl)benzoic acid.

Other aspects of metabolism - The paralytic action and rate of biotransformation in vitro of zoxazolamine was found to be altered in rats bearing certain types of transplantable tumors.[49] Rats transplanted with the Walker 256 carcinosarcoma, in particular, showed highly significant increases, relative to control animals, in the duration of paralysis and in the elevation of levels of zoxazolamine in the brain, liver, and plasma; a reduction in the rate of zoxazolamine metabolism in vitro was also noted.

The pretreatment of rats with desipramine resulted in delayed stomach emptying and, hence, slowed absorption of phenylbutazone, oxyphenbutazone, hydrocortisone, and salicylate.[50] On the other hand, desipramine had no apparent effect on the absorption of orally administered amphetamine or phenobarbital. In another study, the interactions of a variety of acutely and chronically administered contraceptive agents and their effect on pentobarbital narcosis in the rat, as well as on certain metabolic transformations in vitro, were examined.[51] Medroxyprogesterone, alone or in combination with ethynylestradiol, stimulated the metabolism of p-nitroanisole, aminopyrine, and aniline in vitro, although these effects are not as marked as those seen with well-known inducers such as phenobarbital and pesticides.



(XI)

The inhibition of the dealkylation of a variety of drugs by (2,4-dichloro-6-phenylphenoxy)ethylamine (XI) and some of its analogues was reported.[52]

References

1. U. Breyer, Biochem. Pharmacol., 18, 777 (1969).
2. H. S. Posner, R. Culpan, and J. Levine, J. Pharmacol. Exp. Ther., 141, 377 (1963).
3. K. Kanig and U. Breyer, Psychopharmacologia, 14, 211 (1969).
4. D. Krauss, W. Otting, and U. Breyer, J. Pharm. Pharmacol., 21, 808 (1969).
5. J. J. Weir and J. Sanford, J. Pharm. Pharmacol., 21, 169 (1969).
6. J. L. Crammer, B. Scott, and B. Rolfe, Psychopharmacologia, 15, 207 (1969).
7. E. Van Der Kleijn, Arch. Int. Pharmacodyn., 178, 193 (1969).
8. F. Marcucci, R. Fanelli, E. Mussini, and S. Garattini, European J. Pharmacol., 7, 307 (1969).
9. F. J. Dicarlo, M. C. Crew, M. D. Melgar, and L. J. Haynes, J. Pharm. Sci., 58, 960 (1969).
10. D. C. Hobbs, Biochem. Pharmacol., 18, 1941 (1969).
11. A. Jørgensen, V. Hansen, U. D. Larsen, and A. R. Khan, Acta Pharmacol. Toxicol., 27, 301 (1969).
12. W. Soudijn and I. van Wijngaarden, Life Sci., 8, 291 (1969).
13. J. P. P. Heykants, Life Sci., 8, 1029 (1969).
14. K. H. Palmer, B. Martin, B. Baggett, and M. E. Wall, Biochem. Pharmacol., 18, 1845 (1969).
15. M. G. Bogaert, M.-T. Rosseel, and A. F. De Schaepdryver, Arch. Int. Pharmacodyn., 179, 480 (1969).
16. F. J. DiCarlo, M. D. Melgar, L. J. Haynes, R. L. Gala, and M. C. Crew, J. Pharmacol. Exp. Ther., 168, 235 (1969).
17. A. R. Maass, B. Jenkins, Y. Shen, and P. Tannenbaum, Clin. Pharmacol. Ther., 10, 366 (1969).
18. J. W. Bridges, M. R. Kibby, S. R. Walker, and R. T. Williams, Biochem. J., 111, 167 (1969).
19. J. W. Bridges, S. R. Walker, and R. T. Williams, Biochem. J., 111, 173 (1969).
20. J. W. Daniel, Biochem. J., 111, 695 (1969).
21. D. M. Brown, D. P. Hannan, and P. F. Langley, Toxicol. Appl. Pharmacol., 15, 136 (1969).
22. P. A. Harris and S. Riegelman, J. Pharm. Sci., 58, 93 (1969).
23. S. Bailey, P. J. Bunyan, B. D. Rennison, and A. Taylor, Toxicol. Appl. Pharmacol. 14, 13 (1969).
24. J. R. Brown, H. Hughes, and S. Viriyanondha, Toxicol. Appl. Pharmacol., 15, 30 (1969).
25. G. T. Brooks and A. Harrison, Biochem. Pharmacol., 18, 557 (1969).
26. D. I. Cargill, B. G. Steinetz, E. Gosnell, V. L. Beach, A. Meli, G. I. Fujimoto, and B. M. Reynolds, J. Clin. Endocrinol., 29, 1051 (1969).
27. H. Jirku and M. Levitz, J. Clin. Endocrinol., 29, 615 (1969).
28. A. H. Beckett, L. G. Brookes, and E. V. B. Shenoy, J. Pharm. Pharmacol. 21, Suppl., 151S (1969).
29. R. L. Furner, J. S. McCarthy, R. E. Stitzel and M. W. Anders, J. Pharmacol. Exp. Ther., 169, 153 (1969).

30. K. H. Palmer, M. S. Fowler, M. E. Wall, L. S. Rhodes, W. J. Waddell, and B. Bagett, J. Pharmacol. Exp. Ther., 170, 355 (1969).
31. J. S. Robertson and M. Hussain, Biochem. J., 113, 57 (1969).
32. J. E. Sinsheimer and R. V. Smith, Biochem. J., 111, 35 (1969).
33. B. Glasson, A. Benakis, and M. Strolin-Benedetti, Biochem. Pharmacol., 18, 633 (1969).
34. J. J. Kamm, C. R. Ferullo, D. Miller, and E. J. Van Loon, Biochem. Pharmacol., 18, 659 (1969).
35. P. Kabasakalian, M. Taggart, and E. Townley, J. Pharm. Sci., 57, 621 and 856 (1968).
36. A. S. Yard, L. P. Juhasz, and R. M. Grimes, J. Pharmacol. Exp. Ther., 167, 105 (1969).
37. S. F. Sisenwine and S. S. Walkenstein, J. Pharm. Sci., 58, 867 (1969).
38. P. Weiss, R. M. Hersey, C. A. Dujovne, and J. R. Bianchine, Clin. Pharmacol. Ther., 10, 401 (1969).
39. J. Edelson, J. F. Douglas, and B. J. Ludwig, Biochem. Pharmacol., 18, 2331 (1969).
40. P. M. Brotherton, P. Grieveson, and C. McMartin, Clin. Pharmacol. Ther., 10, 505 (1969).
41. K. A. Pittman, D. Rosi, R. Cherniak, A. J. Merola, and W. D. Conway, Biochem. Pharmacol., 18, 1673 (1969).
42. K. Tatsumi, H. Yoshimura, and H. Tsukamoto, Biochem. Pharmacol., 16, 1941 (1967).
43. K. Tatsumi, S. Yoshihara, H. Yoshimura, and H. Tsukamoto, Biochem. Pharmacol., 18, 365 (1969).
44. L. A. Griffiths, Biochem. J., 113, 603 (1969).
45. C.-G. Hammar, W. Hammer, B. Holmstedt, B. Karlen, F. Sjoqvist, and J. Vessman, Biochem. Pharmacol., 18, 1549 (1969).
46. K. Nishikawa, S. Kikuchi, and Z. Suzuoki, Biochem. Pharmacol. 18 1221 (1969).
47. A. H. Beckett and R. D. Hossie, J. Pharm. Pharmacol., 21, 610 and Suppl., 157S (1969).
48. A. M. Guarino, W. D. Conway, and H. M. Fales, European J. Pharmacol., 8, 244 (1969).
49. G. Franchi and R. Rosso, Biochem. Pharmacol., 18, 236 (1969).
50. S. Consolo and S. Garattini, European J. Pharmacol., 6, 322 (1969).
51. A. Jori, A. Bianchetti, and P. E. Prestini, European J. Pharmacol., 7, 196 (1969).
52. R. E. McMahon, J. Mills, H. W. Culp, W. R. Gibson, W. M. Miller, and F. J. Marshall, J. Med. Chem., 12, 207 (1969).

Chapter 23. Structured Water in Biological Systems

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Introduction - Aside from the role of water in biological functions, the structure and properties of water itself continue to fascinate scientists in many research fields. A recent book by Eisenberg and Kauzmann[1] brings together the work of many disciplines. Less detailed discussions are given by Horne[2] and Erlander[3]. In these references, several proposed models of water are discussed. These models are critically evaluated in terms of the X-ray data by Narten and Levy[4]. No single theoretical model for water seems to fit all of the X-ray scattering data. The problem of water structure has been further complicated by the announcement of "anomalous water" by Derjaguin et.al.[5] and the surge of activity relating to its study[6,7,8]. Although anomalous water has already been proposed as a possible form of water in living cells[3], a word of caution is introduced by Franks[9].

The Form and Role of Water in Biological Systems - From the above survey of water, it is apparent that its structure requires much more study. The role that water plays in biological structure and function, and the form in which it exists in these systems are perhaps even more poorly understood. One might start with the statement by Frank[9] to the effect that, "We know next to nothing of the important role that water plays in biochemical phenomena". The outlook is not quite that bleak, but theory is still much more rampant than hard fact. In the area of proteins and peptides, for example, an early paper by Nemethy and Scheraga[10], in which they briefly discuss a tentative relationship between the "ice-likeness" of water and hydrophobic bonding as a factor in protein stabilization, is one of the most widely quoted references in the field despite its tentative nature. Berendsen[11] reiterates that little is known about water's role in biological systems, and then makes a beginning by suggesting a structured water model with pentagonal cages fitted to the helical chains of fibrous proteins. Warner[12] has proposed a theoretical formulation for peptides and proteins that permits a hydrogen-bonded interaction between water structured in an ice-like lattice and a hexagonal arrangement of the peptide oxygens, as well as other useful stabilizing interactions. Each of these theoretical considerations has implied a condition in which the water was in some way and shape bound to the protein. Other speculations are continuing. In a determination of the quantity of "bound water" in proteins, a recent study by Bull and Breese[13] suggests that at 25° C., saturation of the polar residues of solid proteins occurs at 0.92 relative humidity with 0.3 g of water per gram of protein. They obtained similar results with ten different proteins, and these general values are in line with those obtained by previous workers. In attempting to correlate this quantity of "bound water" with the number of polar groups (minus the amides), as if the polar residues were the only ones involved, they come up with a value of six moles of bound water per polar residue. However, the manner in which bound water is disposed throughout a protein molecule is still a matter for considerable dispute. Goto and Isemura[14]

have indicated that, in peptides with non-polar side chains such as diglycine, triglycine, and tetraglycine, the peptide bonds are also hydrated. Such peptides, of course, do have N-terminal and C-terminal polar groups which could account for some water binding in the Bull and Breese[13] manner, but the important consideration is that the hydration number increases by one for each additional peptide bond introduced in the series. Assarsson and Eirich[15], by a study of density changes and absolute viscosity measurements, indicate that the fully substituted peptide dipole (as judged from N,N-disubstituted amide models) most likely has two water molecules bonded to the amide carbonyl and one water weakly associated with the nitrogen atom. With monosubstituted amides the method is less definitive. Here the amide itself is capable of intermolecular hydrogen bonding, which is merely replaced by amide-water bonding as dilution proceeds. However, here, too, there is a definite change in viscosity at the 1:1-water:amide molar ratio as well as additional perturbations indicating higher ratios of association. These results suggest that the peptide linkage is a possible site of water binding in proteins. Warner[16] in seeking support for his protein model has calculated the quantity of bound water for the tobacco mosaic virus proposed structure, arriving at a value of about 32% when all peptide carbonyls and hexagonal centers are occupied. However, the true nature of the bound water association still remains to be proved.

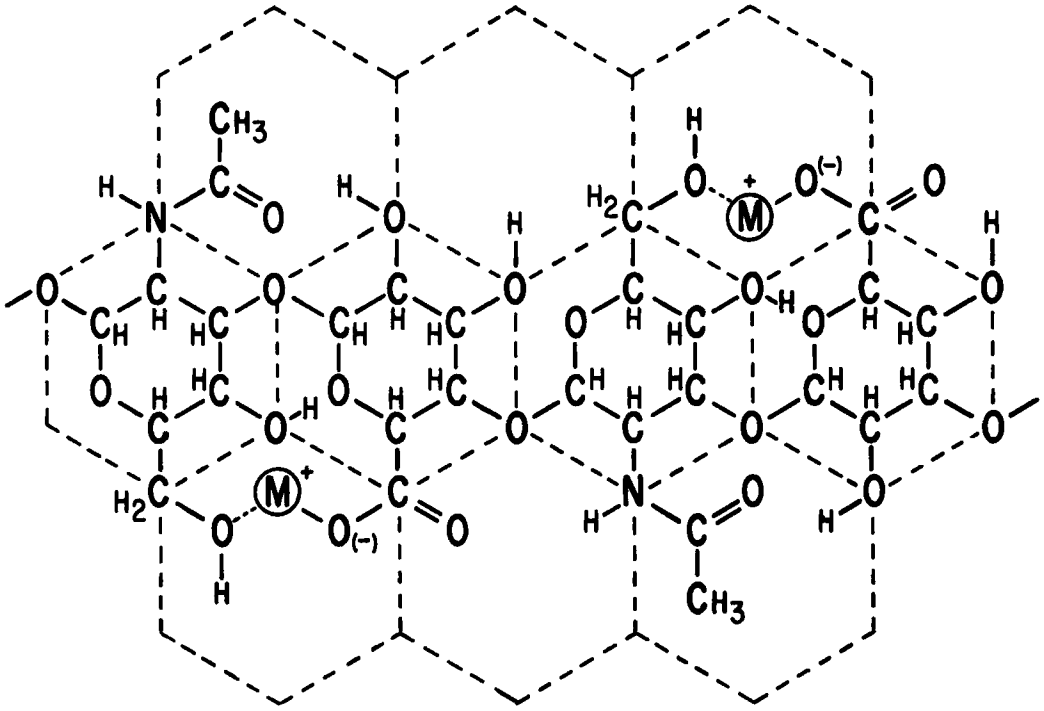
In addition to speculations about the form of water in proteins, workers have turned their theoretical considerations to other biological macromolecules. As early as 1953, Jacobson[17] showed a remarkable fitting of the ice lattice to the Watson-Crick model of DNA. The same thing can be shown with a polysaccharide such as hyaluronic acid (Figure 1). Vandenheuvel[18] has studied similar interactions between lipid-water layers with molecular models.

Other studies relating to the interaction of water with smaller organic molecules of biological importance include an infrared examination of chlorophyll-water interactions by Ballschmeter and Katz[19]. This study implicates water in the binding of two chlorophyll molecules to each other, with the water coordinated with the magnesium of one molecule and hydrogen bonded to two carbonyl oxygens of the other. It should be noted that the drawing of the chlorophyll-water interaction (in their Figure 15) as judged by Dreiding Stereomodels involves two carbonyl oxygens which are about 2.9 A apart, equivalent to a "first neighbor" water distance. To bridge one water molecule between them as illustrated requires considerable distortion of the H-O-H angle or else a decidedly non-linear -O-H---O hydrogen bond.

The crystal and molecular structure of β-adenosine-2'-β-uridine-5'-phosphoric acid precipitated from aqueous solution[20] shows that water molecules serve as a major part of the binding force between the nucleotide molecules. In this instance hydrogen bonds do not directly link the bases together. Webb and Bhorjee[21], using infrared measurements to study factors influencing viability and conformation, reiterate the importance of water in maintaining an organized structure for DNA from *E. coli*.

Figure 1 - Hyaluronic Acid in Water Lattice
(Warner, previously unpublished figure)

----Outline of "First neighbor" oxygen network of ice
Ⓜ = possible monovalent metal



Their work indicates the possibility of also substituting myo-inositol for this water requirement. (A feasible explanation for this replacement is suggested in a later section).

For those desiring more detail on the "Forms of Water in Biologic Systems" prior to about 1965, a special volume sponsored by the New York Academy of Sciences[22] is recommended.

Nuclear Magnetic Resonance in the Study of Water Structure in Biological Systems - Additional work has been done by Chapman and McLauchlan[23] on the hydration structure of collagen using N.M.R. and dielectric measurements. Questioning Berendsen's[11] idea of specific water-collagen interactions, they suggest that water in a confined space of molecular magnitude, such as between collagen strands, simply forms a hydrogen-bonded straight chain structure, independent of specific interactions with the channel walls. However, if the peptide carbonyls lining these spaces are hydrophilic groups[14], it is difficult to see how this independence could be maintained.

Other important applications of N.M.R. concern a study of the state of

water in tissues such as muscle, brain and nerve. In line with the earlier conclusions of Bratton, Hopkins and Weinberg[24] that a fraction of the intracellular water in muscle has a restricted rotational freedom, additional experiments by Hazlewood, Nichols, and Chamberlain[25] suggest that this water may exist in at least two ordered states in skeletal muscle. The restriction of motional freedom of this water is thought to be caused by association with cellular macromolecules. The latter authors also demonstrate that an agar gel reduces motional freedom of water, and that the effect is reversible with heating and cooling. Independent verification for structured water in muscle and brain appeared in a simultaneous publication by Cope[26]. He suggests that in general "tissue water has a significantly greater degree of crystallinity than liquid water". Here again two fractions are implied, one of which is more highly structured than the other. The structuring effect of agar was also verified, but gelatin shows no structuring effects. The state of water in frog nerves is being studied by Swift and Fritz[27], and they find accessible and inaccessible regions to paramagnetic ions by the use of N.M.R.

Although there are no positive indications that structured water plays a role in the mechanism of muscle contraction, a theoretical mechanism employing the ice lattice can be devised. Such a scheme uses protein-water interactions in two phases involving interchanging enol and keto forms of the peptide carbonyls, and was first mentioned as a possible contraction mechanism in an informal discussion[28]. A complete elaboration of this concept as a model for muscle contraction has been published[29]. This model, which employs water in an active structural and mechanical sense, may be relevant to the earlier observation of Goodall[30] that proton transfer could be the rate-determining step in muscle contraction. Horne and Johnson[31] have shown that variables such as pressure, temperature, and isotope (D_2O) may influence proton transfer in the aqueous environment, and each of these variables has been shown to have an effect on muscle contraction.

The Importance of Water Structure in Anesthesia - One of the current theories of anesthesia invokes hydrate structures of the clatharate type, building up around the anesthetic agent in the water, as the prime cause of anesthesia. The essential parts of this theory were presented independently by Miller[32] and Pauling[33]. Studies to substantiate or negate this theory are still in progress. A very concise summary of the clatharate hydrate theory is presented by Miller[34]. Additional work to overcome objections to this theory yielded the tentative conclusion that the correlation of anesthetic potency with lipid solubility is slightly superior to the correlation with hydrate dissociation pressure[35]. The clatharate hydrates and deuteriohydrates of the anesthetic, cyclopropane, have been prepared[36], and these compounds are stable only at low temperature. An attempt to study the feasibility of clatharate formation under conditions that might be considered as physiological has been evaluated by X-ray diffraction. One of the tetra-n-butylammonium halides, which seem to readily form clatharates in water at high hydrostatic pressure[37], when examined by X-ray diffraction in concentrated aqueous solution at ordinary pressures[38] shows a radial distribution function that is in good agreement

for the compound inserted in an ice-I lattice and in poor agreement with calculations based on the gas hydrate model. This may indicate that elevated pressure and low temperature are required for clatharate formation.

The effect of cyclopropane anesthesia on tritiated water flux across the gut is another attempt to study the influence of anesthesia on water structure[39]. High concentrations of cyclopropane produce tissue damage, but no change in water flux across the rat cecum. Moderate concentrations produce minimal tissue damage but some slowing of water flux across the membrane was noted. It is suggested that this experiment is consistent with the hydrate theory of anesthesia, but not necessarily a direct proof of this mechanism of operation of the anesthetic.

The Possible Involvement of Structured Water in the Reaction Mechanism of Pharmacologic Agents - As Miller[34] says, there are no "unequivocal demonstrations of the importance of water structure on the mechanism of action of pharmacologic agents, but it would be surprising if the water played no role at all". The problem of proving its role relates in part to our inability thus far to clearly define water structure in either its liquid state or its biological context. However, it is possible to start with the assumption that water in biological media may exist in an ice-like lattice or some other selected form, and then to make some comparisons between this lattice and pharmacologic agents in a three-dimensional sense. The work of Grant and Alburn[40] demonstrating enhanced enzyme and other reactions in frozen aqueous systems, has called attention to the possible role of ice as a model for biological "structured water" systems, though not necessarily proving this possibility.

The studies to be described were made by Warner[28,41] with Dreiding Stereomodels and employ the ice lattice as the selected form of water for the comparisons. The cyclitol sugar, scyllo-inositol, when inserted into an ice lattice, has the six oxygens of its equatorial hydroxyls in a nearly identical pattern and position to six oxygens of the ice lattice[41]. Biologically, Magasanik and Chargoff[42] observed many years ago that scyllo-inositol is not oxidized by Acetobacter suboxydans. Myo-inositol, an isomer having one axial and five equatorial hydroxyls, is oxidized to a monoketone, with the axial hydroxyl being specifically oxidized. In the sugar-ice lattice model study, the single axial hydroxyl of myo-inositol is the only oxygen that does not orient itself with an oxygen position in the ice lattice. The oxidized product, the monoketone, again has a keto oxygen which is fairly close to an oxygen position in the ice lattice.

The close correspondence of many of the hydroxyl groups in sugars with oxygen positions in the ice lattice probably correlates best with water at certain temperatures (that is, an ice lattice with slightly expanded dimensions to fit water oxygen distances at biological temperatures). The possible ability of substances like glycerol or sugars, with covalently separated oxygens, to stabilize biological systems at low temperatures where water distances might not "fit" has been alluded to[41]. In the work of Webb already noted[21] in which he found that myo-inositol could replace the water requirement under dehydrating conditions, the close fitting

between hydroxyl spacings in the sugar and oxygen spacings in the ice lattice arrangement of water could be fundamental to the effectiveness of the replacement. A rigid covalent ring system like that of the inositols, might be quite resistant to oxygen-oxygen distance changes over a wide temperature range, whereas water distances could be varying continuously and be suitable only in the biological temperature range.

Larger molecules such as steroids may also be fitted into an ice lattice and studied with molecular models. This has been done with hydrocortisone[43] and the implications of the possible interactions are discussed in detail. Even the non-polar methyl groups may be extremely important in influencing perturbations in the surrounding water lattice. The implication is that if a biological system is initially in a state of equilibrium with an encompassing water lattice, then the entrance of a substrate produces specific perturbations in this lattice at specific sites. This could constitute the first step in reactivity of a substrate.

The use of molecular models to show possible water-substrate fittings for biotin, quinones and several other structures has been published[43]. Even the conjugated double bonds in the polyunsaturated side chain of coenzyme Q_{10} are compatible with the second neighbor oxygen distance in an ice lattice[44]. Theoretically water should be capable of systematically interacting with this ordered chain of electron donating sites.

If the water in a biological environment is indeed present in an ice-like lattice, then the possible consequence of substituting or perhaps "permeating" this bound water around macromolecules with a substrate or drug may be thought of in a general way such as the following: A peptide carbonyl group, for example, of the macromolecule is in hydrogen-bonded contact with surrounding water. Since water molecules have hydrogen atoms readily available, the hydrogen bond would involve the arrangement -NH-C=O ---H-O-H. If a substrate or drug bearing a keto group, for example, now moves into the general area, this keto group could not normally furnish a hydrogen to establish a hydrogen bond with the peptide carbonyl group. The situation might be alleviated, however, if the peptide bond were enolized. With this change it could furnish the necessary hydrogen for the bonding, but the net result would be a change in the protein structure which might be thought of as "substrate-induced". Alternately, the bonding could perhaps in some instances be established if the keto group were capable of enolizing by way of the hydrogen on its α-carbon atom to institute a "protein-induced" substrate change. As a final consideration, one might suggest that an ether oxygen would be equally capable of inducing a "substrate-induced" protein change, but might have a different degree of stability to a "protein-induced" substrate change. This very fragmentary exposition is further detailed in references 28, 41 and 43.

Many of the molecules referred to above have rigid structures with the reactive groups attached to a stable framework, and it is relatively easy to assign the position of reactive groups in the ice lattice. One variable that has been studied here as far as the ice is concerned is to

examine the fittings in both the hexagonal and diamond cubic form of ice. These two forms are briefly described by Dowell and Rinfret[45]. With larger molecules like steroids which interact with more than one layer of the ice lattice, different relationships may be noted. The study of flexible structures such as triglycerides or open chain alkyl compounds in an ice lattice raises some uncertainties about group placement, and it is more difficult to achieve a unique fitting.

Direct experimental evidence that pharmacologic agents have an influence on structured or bound water comes from the somewhat unlikely area of studies with flour/water doughs in a paper by Tracey[46]. Central nervous system stimulants and depressives have profound effects on the physical properties of these doughs. This paper is also a stimulating essay review on several other facets of the implications of water in biology.

Many proteins and especially small cyclic peptides are potent pharmacologic agents. Since the proteins and peptides are also flexible chains, different theoretical chain conformations are possible and the names of these are now practically household words. Possible relationships between some of these protein conformations and molecular models of an ice lattice have been explored. The spacing of peptide oxygens on an α-helix is not consistently compatible with the spacings in an ice lattice, and only accidental correlations are to be expected. The spacings of peptide oxygens in the pleated sheet or β-structure can come quite close to some of the spacings in an ice lattice. Vandenheuvel[47] has exploited this in a model study of membrane systems. His model does not include attachment to the complete lattice system. One theoretical protein conformation that does attempt to make a complete point-by-point hydrogen bonded attachment of the ice lattice to the protein chain is the conformation first described by Warner[48]. For easy reference this may be called the "hexagonal conformation", an allusion to the hexagonal arrangement of the peptide oxygens which quite precisely matches and in theory at least can be bonded to a similar hexagonal network in the ice lattice. The general concept of this theory, some of the objections to it and its possible extensions were reported in a recent symposium[44]. This conformation, together with the large number of protein sequences now available, has permitted the formulation of some theoretical models for peptide and protein molecules which can be tested experimentally and also checked against existing data. In the case of TMV protein[16], some good correlations were achieved between existing experimental data on the size and shape of the rod and the theoretically derived model. For the valinomycin model[44], the theoretical concept is not consistent with the X-ray diffraction studies on the valinomycin-$KAuCl_4$ complex[49]. In fairness to the theoretical model, it should be mentioned that the model was proposed as a preferred structure for an aqueous environment at biological temperatures, whereas the crystals examined by X-ray were grown from solutions of the complex in equal mixtures of chloroform and xylene.

The proposed utility of the hexagonal concept of protein conformation in a muscle contraction mechanism has already been noted[29]. With the large body of X-ray data now becoming available on intact striated muscle[50],

this structured water model should be amenable to rigorous evaluation. Another paper[51] describes the attempted application of the hexagonal concept to proteins containing cystines and cysteines. To select one example from that paper, scenedesmus ferredoxin protein[52] has a primary sequence of 96 amino acids with six cysteines at residues 18, 39, 44, 47, 77 and 85. When these residue positions are laid out on a hexagonal spiral (similar to that used for TMV protein[16]) then the six cysteines are all found in a very compact area in one portion of the subunit. Neither the α-helix nor the pleated sheet would produce a similar degree of clustering of these rather widely separated primary sequence residues. Whether the clustering of these -SH groups is necessary for reactivity is not known for certain.

Summary - It is apparent from our brief survey that large deficiencies exist in our knowledge of water itself and its structural role in biology. Certain theoretical approaches are interesting but the experiments to verify or disprove the theories still need to be done. Much of the information about the role of water tends to be hidden in brief sections of papers whose main topic is some other problem. The writer apologizes for many instances where such interesting information has no doubt been overlooked. It is hoped that enough of the recent information has been included so that more people may be inspired to consider the role of structured water in biologic systems and in pharmacologic function.

REFERENCES

1. D. Eisenberg and W. Kauzmann, "The Structure and Properties of Water", Oxford Univ. Press, New York (1969).
2. R. A. Horne, "The Structure of Water and Aqueous Solutions", in "Survey of Progress in Chemistry", ed: A. F. Scott, Vol. 4, pp. 1-43, Academic Press, New York (1968).
3. S. R. Erlander, Sci. J., 5A, 60 (1969).
4. A. H. Narten and H. A. Levy, Science, 165, 447 (1969).
5. B. V. Derjaguin, N. V. Churaev, N. N. Fedyakin, M. V. Talaev and I. G. Ershova, Izv. Akad. Nauk. SSSR, 10, 2178 (1967).
6. E. Willis, G. K. Rennie, C. Smart and B. A. Pethica, Nature, (London), 222, 159 (1969).
7. L. J. Bellamy, A. R. Osborn, E. R. Lippincott and A. R. Bandy, Chem. Indust., 688 (May, 1969).
8. W. Hertl and M. L. Hair, Nature, (London), 223, 1150 (1969).
9. F. Franks, Chem. Brit., 5, 420 (1969).
10. G. Nemethy and H. A. Scheraga, J. Phys. Chem., 66, 1773 (1962).
11. H. J. C. Berendsen, Fed. Proc., Fed. Am. Soc. Exp. Biol., 25, 971 (1966).
12. D. T. Warner, J. Theor. Biol., 1, 514 (1961).
13. H. B. Bull and K. Breese, Arch. Biochem. Biophys., 128, 488 (1968).
14. S. Goto and T. Isemura, Bull. Chem. Soc. Jap., 37, 1697 (1964).
15. P. Assarsson and F. R. Eirich, J. Phys. Chem., 72, 2710 (1968).
16. D. T. Warner, J. Theor. Biol., 6, 118 (1964).
17. B. Jacobson, Nature, (London), 172, 666 (1953).
18. F. A. Vandenheuvel, J. Amer. Oil Chem. Soc., 43, 258 (1966).
19. K. Ballschmeter and J. J. Katz, J. Amer. Chem. Soc., 91, 2261 (1969).
20. E. Shefter, M. Barlow, R. A. Sparks and K. N. Trueblood, Acta Crystallogr. Sect. B, 25, 895 (1969).
21. S. J. Webb and J. S. Bhorjee, Can. J. Biochem., 46, 691 (1968).
22. Ann. N. Y. Acad. Sci., 125, Art. 2, 249-772 (1965), "Forms of Water in Biologic Systems", ed: H. E. Whipple.
23. G. E. Chapman and K. A. McLauchlan, Proc. Roy. Soc., London, Ser. B., 173, 223 (1969).
24. C. B. Bratton, A. L. Hopkins and J. W. Weinberg, Science, 147, 738 (1964).
25. C. F. Hazlewood, B. L. Nichols and N. F. Chamberlain, Nature (London), 222, 747 (1969).
26. F. W. Cope, Biophys. J., 9, 303 (1969).
27. T. J. Swift and O. G. Fritz, Jr., Biophys. J., 9, 54 (1969).
28. D. T. Warner, Reference 22, p. 769.
29. D. T. Warner, J. Theor. Biol., 26, 289 (1970).
30. M. C. Goodall, Nature (London), 182, 677 (1958).
31. R. A. Horne and D. S. Johnson, Nature, (London), 209, 82 (1966).
32. S. L. Miller, Proc. Nat. Acad. Sci., 47, 1515 (1961).
33. L. Pauling, Science, 134, 15 (1961).
34. S. L. Miller, Fed. Proc., Fed. Amer. Soc. Exp. Biol., 27, 879 (1968).
35. E. I. Eger, C. Lundgren, S. L. Miller and W. C. Stevens, Anesthesiology, 30, 129 (1969).
36. D. R. Hafemann and S. L. Miller, J. Phys. Chem., 73, 1392, 1398 (1969).

37. R. A. Horne and R. P. Young, J. Phys. Chem., 72, 376 (1968).
38. A. H. Narten and S. Lindenbaum, J. Chem. Phys., 51, 1108 (1969).
39. E. Y. Berger, F. R. Pecikyan and G. Kanzaki, Amer. J. Physiol., 217, 414 (1969).
40. N. H. Grant and H. E. Alburn, Arch. Biochem. Biophys., 118, 292 (1967).
41. D. T. Warner, Nature, (London), 196, 1055 (1962).
42. B. Magasanik and E. Chargaff, J. Biol. Chem., 174, 173 (1948).
43. D. T. Warner, Reference 22, 605 (1965).
44. D. T. Warner, J. Amer. Oil Chem. Soc., 44, 593 (1967).
45. L. G. Dowell and A. P. Rinfret, Nature, (London), 188, 1144 (1960).
46. M. V. Tracey, Proc. Roy. Soc., London, Ser. B., 171, 59 (1968).
47. F. A. Vandenheuvel, J. Amer. Oil Chem. Soc., 42, 490 (1965).
48. D. T. Warner, Nature, (London), 190, 120 (1961).
49. M. Pinkerton, L. K. Steinrauf and P. Dawkins, Biochem. Biophys. Res. Commun., 35, 512 (1969).
50. H. E. Huxley and W. Brown, J. Mol. Biol., 30, 383 (1967).
51. D. T. Warner, J. Theor. Biol., in press.
52. K. Sugeno and H. Matsubara, Biochem. Biophys. Res. Commun., 32, 951 (1968).

Chapter 24. Structure and Biological Activity Interrelationships in Peptides

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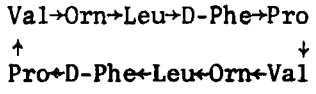
Introduction - The isolation of urease from jack beans by Sumner in 1926 marked a turning point in the history of protein chemistry. The full significance of the event was not immediately recognized: for the organic chemists of those days, it was inconceivable that an enzyme could have protein structure or a protein could possess enzymic activity. The absence of reactive groups in proteins made it difficult to understand that they can act as catalysts with an almost miraculous enhancement of reaction rates and with amazing substrate specificity. Today we accept without hesitation that all enzymes are proteins. The impact of x-ray crystallography led to a general image of biologically active proteins: complex structures with well-defined architecture, with a niche in myoglobin and haemoglobin for the oxygen molecule to be carried, with clefts in enzymes which first have to accomodate a specific substrate and then operate on it. A remarkable early insight into this relation between architecture and biological activity was demonstrated by du Vigneaud who in about 1950 wrote: "Some investigators have been inclined to conclude that one or two disulfide linkages have a special function in insulin. Our own tendency was, and still is, to regard the architecture of the molecule as a whole as the important factor with regard to its hypoglycemic action..."

The close relationship between biological activity and molecular architecture of proteins is by now generally accepted. Yet, while the existence of well defined geometry of proteins is beyond question, until recently the general impression about peptide conformation was that of randomness. The present review intends to demonstrate that the importance of conformation (architecture) of biologically active peptides gradually emerges and receives considerable attention.

Architecture of Microbial Peptides - The cyclic character is the earliest recognized general feature of peptide antibiotics.[1] Open chain peptide antibiotics are rare exceptions (gramicidin A,[2] bottromycin[3]). The best studied example of peptide antibiotics, gramicidin S (I), was recently the

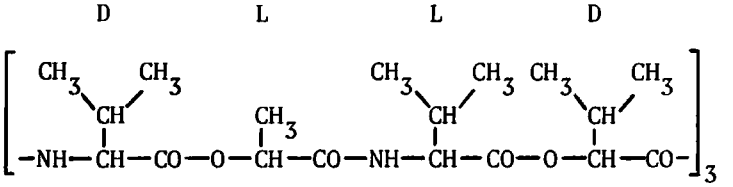


I

subject of intensive studies, oriented toward the conformation of this homodetic[4] cyclic peptide. The serious limitations caused by the ring structure and by the planarity of the peptide bond still allow several possible arrangements of the peptide backbone and the amino acid side chains. The conformation of gramicidin S was approached through theoreti-

cal calculations.[5,6] A series of nmr studies[7-10] provided convincing evidence for an antiparallel-sheet conformation.

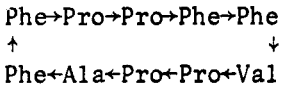
The true biological role of microbial peptides is probably not their inhibitory action against other organisms. Such compounds should have some function in the cells which produce them. The function is mostly unknown so far, yet a possible role for microbial peptides could be that of carriers of specific "substrates" through the lipid-rich membranes of microbial cell walls. A remarkable example is valinomycin (II), which can



II

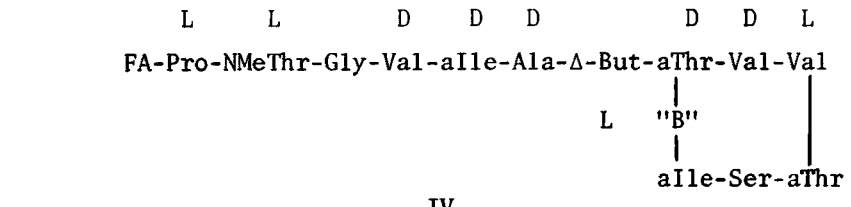
accomodate a K^+ ion in its ring.[11] In the presence of valinomycin potassium ions can pass through such membranes 10,000 times faster than sodium ions. The expression "ionofore" was conceived[12] for compounds with similar functions. The carrier role of microbial peptides can serve as explanation for their cyclic architecture.

The conformational features of ring-structure,[1] hypercyclization,[13] the presence of N-methyl amino acids and other imino acids[14] are not the attributes of peptide antibiotics only. The toxic principles of Amanita phalloides, phalloidin[15] and amanitin[15] exhibit similar characteristics and the more recently discovered and synthesized component of the same fungus antaminid[16,17] (III), a peptide which can antagonize the toxic effects of amanitin, is also cyclic. In this particular case no D-amino

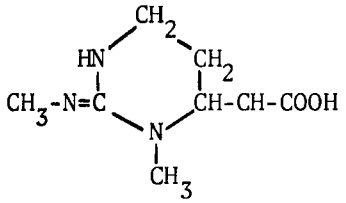


III

acids participate in the sequence. The usual explanation that the presence of one or more D-amino acids facilitates ring closure still could be valid. In antamanid four proline residues might act in the same way. That proline rich peptides cyclize more readily is shown by the formation of cyclotriproline[18] in good yield. It is interesting to note, however, that not less than 7 D-amino acids occur in the peptide antibiotic stendomycin[19] (IV) for which a preferred conformation with the hydrophobic amino acid side chains on the outer surface of the molecule and a possible carrier role were postulated.[20]



IV

B = 

aIle = alloisoleucine
aThr = allothreonine
Δ-But = dehydrobutyrine
FA = Fatty acids
(e.g. isomyristic acid)

Architecture of Peptide Hormones - The existence of a preferred conformation of cyclic peptides such as oxytocin or vasopressin can a priori be expected. The existence or significance of a preferred conformation is less obvious in the case of open chain peptide hormones. It is somewhat surprising, therefore, to observe the optical rotatory dispersion (ORD) and circular dichroism (CD) spectra[21] of the gastrointestinal hormone (porcine) secretin[22] (V). The spectra show striking similarity with those of lysozyme, a protein with low but well established helix content. The

His	Ser	Asp	Gly	Thr	Phe	Thr	Ser	Glu	Leu	Ser	Arg	Leu	Arg
1	2	3	4	5	6	7	8	9	10	11	12	13	14

Asp	Ser	Ala	Arg	Leu	Gln	Arg	Leu	Leu	Gln	Gly	Leu	Val	NH_2
15	16	17	18	19	20	21	22	23	24	25	26	27	

V

ORD and CD spectra of secretin were interpreted as indications for a part of the molecule being in a rigid form. A comparison of the spectra of the hormone itself with the spectra of a series of shorter peptides corresponding to partial sequences of secretin suggested that this rigidity in one part of the chain is a consequence of intramolecular cooperative interactions between distant parts of the molecule.

While the spectra of the shorter fragments from secretin do not show complete randomness, they either represent some preferred conformation for which no simple geometric description can be formulated, or they may correspond to several conformations simultaneously present in the population of individual molecules. Certain rigidity, however, could be present even in small peptides. The sequence of bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) is suggestive in this respect because this nonapeptide con-

tains three L-proline residues, two of them neighbors. Yet the ORD and CD spectra of bradykinin[23] failed to reveal characteristics that would point to helical or other well defined conformations. This observation is in harmony with the diffusion studies of Craig[24] which also suggest considerable conformational freedom for bradykinin.

The conclusion that a peptide such as bradykinin consisting of nine amino acids does not have a preferred conformation but that a well defined geometry develops from a certain chain length on (secretin contains twenty-seven amino acids, glucagon with fairly similar sequence and somewhat similar spectra[25] twenty-nine amino acids), would be attractive but is not well founded. Examination of the ORD and CD spectra[23] of the intestinal hormone cholecystokinin-pancreozymin (CCK),[26] a single chain peptide of 33 amino acids, gives no evidence for structural rigidity which would express itself in spectra similar to those of helical molecules.

The contrast between the presence of well defined conformation in secretin and the absence of rigid geometry in bradykinin or CCK prompts the speculation that the cooperative intramolecular interactions which determine the architecture of secretin might be replaced by similar but intermolecular interactions in CCK (perhaps also in bradykinin), that is by interactions between the hormone and the yet unknown receptor site. Some support for this speculation can be found in the remarkable fact that while with secretin practically the whole chain is needed for full biological activity, this is not true for CCK: already relatively short C-terminal sequences reveal the various hormonal activities of the CCK itself.[27]

Similarly in gastrin[28-31] a short C-terminal part of the heptadecapeptide sequence is sufficient for activity.[32,33] Some of the cooperative interactions determining preferred conformations result in hydrophobic bonds[34] which in turn through the exclusion of water can stabilize or organize a potentially helical stretch. Sequences with hydrophobic amino acid residues at each third or fourth position were recognized by Perutz, Kendrew and Watson[35] in the helical regions of haemoglobin. The potential helix in these sequences forms a real helix in which the hydrophobic amino acids are on one side of the helical region and in contact with a non-polar region of the molecule. This non-polar region can be quite distant in terms of amino acid sequence, but is brought into proximity by an appropriate folding of the backbone. A similar interaction, perhaps intermolecular, is suggested by the distribution of the amino acids with hydrophobic side chains in the peptide hormone calcitonin.[36-39] The amino acid sequence of the hormone changes significantly from species to species (Fig. 1), yet the position of the non-polar residues remains unchanged during evolution. In all the different calcitonins positions 4, 9, 12, 16, 19, 22 and 27 are occupied by leucine, phenylalanine or tyrosine residues and these three amino acids occur only in the here mentioned positions. This distribution of hydrophobic side chains gives strong support to the thought that the "reaction" between hormone and receptor consists of the stabilization (formation, organization) of regions with well determined architecture.

Calcitonins

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Salmon	Cys	Ser	Asn	Leu	Ser	Thr	Cys	Val	Leu	Gly	Lys	Leu	Ser	Gln	Glu	Leu
Sheep	Cys	Ser	Asn	Leu	Ser	Thr	Cys	Val	Leu	Ser	Ala	Tyr	Trp	Lys	Asp	Leu
Pig	Cys	Ser	Asn	Leu	Ser	Thr	Cys	Val	Leu	Ser	Ala	Tyr	Trp	Arg	Asn	Leu
Man	Cys	Gly	Asn	Leu	Ser	Thr	Cys	Met	Leu	Gly	Thr	Tyr	Thr	Gln	Asp	Phe

	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Salmon	His	Lys	Leu	Gln	Thr	Tyr	Pro	Arg	Thr	Asn	Thr	Gly	Ser	Gly	Thr	Pro-NH_2
Sheep	Asn	Asn	Tyr	His	Arg	Tyr	Ser	Gly	Met	Gly	Phe	Gly	Pro	Glu	Thr	Pro-NH_2
Pig	Asn	Asn	Phe	His	Arg	Phe	Ser	Gly	Met	Gly	Phe	Gly	Pro	Glu	Thr	Pro-NH_2
Man	Asn	Lys	Phe	His	Thr	Phe	Pro	Gln	Thr	Ala	Ile	Gly	Val	Gly	Ala	Pro-NH_2

Figure 1

References

1. E. P. Abraham, Biochemistry of Some Peptide and Steroid Antibiotics (Wiley, New York, 1957).
2. R. Sarges and B. Witkop, J. Am. Chem. Soc., 86, 1862 (1964).
3. S. Nakamura, T. Shikaike, H. Yonehara and H. Umezawa, J. Antibiotics, Ser. A, 18, 61 (1965).
4. R. Schwyzer, B. Iselin, W. Rittel and P. Sieber, Helv. Chim. Acta, 39, 872 (1956); R. Schwyzer, Chimia, 12, 58 (1958).
5. A. M. Liquori, P. DeSantis, A. L. Kovacs and L. Mazzarella, Nature, 211, 1039 (1966).
6. B. Vanderkooi, S. J. Leach, G. Nemethy, R. A. Scott and H. A. Scheraga, Biochemistry, 5, 2991 (1966).
7. F. Quadrifoglio and D. W. Urry, Biochem. Biophys. Res. Commun., 29, 785 (1967).
8. A. M. Liquori and F. Conti, Nature, 217, 635 (1968).
9. A. Stern, W. A. Gibbons and L. C. Craig, Proc. Nat. Acad. Sci., 61, 734 (1968).
10. R. Schwyzer and U. Ludescher, Helv. Chim. Acta, 52, 2033 (1969).
11. T. E. Andreoli, M. Tieffenberg and D. C. Tosteson, J. Gen. Physiol., 50, 2527 (1967); V. T. Ivanov, I. A. Laine, N. D. Abdulaev, L. B. Senyavina, E. M. Popov, Yu. A. Ovchinnikov and M. M. Shemyakin, Biochem. Biophys. Res. Commun., 34, 803 (1969); M. Pinkerton, L. K. Steinrauf and P. Dawkins, Biochem. Biophys. Res. Commun., 35, 512 (1969).
12. B. C. Pressman, E. J. Harris, W. S. Jagger and J. H. Johnson, Proc. Nat. Acad. Sci., 58, 1949 (1967).
13. M. Bodanszky and D. Perlman, Antimicrobial Agents & Chemotherapy, 1967, p. 464 (1968).
14. D. Perlman and M. Bodanszky, Antimicrobial Agents & Chemotherapy, 1965, p. 122 (1966).

15. T. Wieland, Progress in the Chemistry of Organic Natural Products, Vol. 25, p. 214 (Springer-Verlag, New York, 1967).
16. T. Wieland, G. Luben, H. Ottenheym, J. Faesel, J. X. DeVries, A. Prox and J. Schmid, Angew. Chem. Internat. Edit., 7, 204 (1968).
17. A. Prox, J. Schmid and H. Ottenheym, Liebigs Ann. Chem., 722, 179 (1969).
18. M. Rothe, D. Steffen and I. Rothe, Angew. Chem., 77, 347 (1965).
19. M. Bodanszky, J. Izdebski and I. Muramatsu, J. Am. Chem. Soc., 91, 2351 (1969).
20. M. Bodanszky and A. Bodanszky, Nature, 220, 73 (1968).
21. A. Bodanszky, M. A. Ondetti, V. Mutt and M. Bodanszky, J. Am. Chem. Soc., 91, 944 (1969).
22. V. Mutt and J. E. Jorpes, Pharmacology of Hormonal Polypeptides and Proteins, N. Back, L. Marini and R. Paoletti, Ed., p. 569 (Plenum Press, New York, 1968).
23. A. Bodanszky, M. Bodanszky, J. E. Jorpes, V. Mutt and M. A. Ondetti, in preparation.
24. L. C. Craig, E. J. Harfenist and A. C. Paladini, Biochemistry, 3, 764 (1964).
25. W. B. Gratzer, E. Baily and G. H. Beaven, Biochem. Biophys. Res. Commun., 28, 914 (1967); W. B. Gratzer, G. H. Beaven, H. W. E. Rattle and E. M. Bradbury, European J. Biochem., 3, 276 (1968).
26. J. E. Jorpes and V. Mutt, Acta Chem. Scand., 18, 2408 (1964); V. Mutt and J. E. Jorpes, European J. Biochem., 6, 156 (1968).
27. M. A. Ondetti, J. T. Sheehan and M. Bodanszky, Pharmacology of Hormonal Polypeptides and Proteins, p. 18 (Plenum Press, New York, 1968).
28. H. Gregory, P. M. Hardy, D. S. Jones, G. W. Kenner and R. C. Sheppard, Nature, 204, 941 (1964).
29. R. A. Gregory, H. J. Tracy and M. I. Grossman, Nature, 209, 583 (1966); P. H. Bentley, G. W. Kenner and R. C. Sheppard, Nature, 209, 583 (1966).
30. K. L. Agarwal, J. Beacham, P. H. Bentley, R. A. Gregory, G. W. Kenner, R. C. Sheppard and H. J. Tracy, Nature, 219, 614 (1968).
31. K. L. Agarwal, G. W. Kenner and R. C. Sheppard, Experientia, 25, 346 (1969).
32. J. S. Morley, Proc. Roy. Soc. B., 170, 97 (1968).
33. J. S. Morley, Federation Proceedings, 27, 1314 (1968).
34. W. Kauzmann, Advanc. Protein Chem., 14, 1 (1959).
35. M. F. Perutz, J. C. Kendrew and H. C. Watson, J. Mol. Biol., 13, 669 (1965).
36. H. D. Niall, H. T. Keutmann, D. H. Copp and J. T. Potts, Proc. Nat. Acad. Sci., 64, 771 (1969).
37. R. Sauer, H. D. Niall and J. T. Potts, Jr., personal communication.
38. J. T. Potts, Jr., H. D. Niall, H. T. Keutmann, H. B. Brewer, Jr., and L. J. Deftos, Proc. Nat. Acad. Sci., 59, 1321 (1968); P. H. Bell, W. F. Barg, Jr., D. F. Colucci, M. C. Davies, C. Dziobrowski, M. E. Englert, E. Heyder, R. Paul and E. H. Snedeker, J. Am. Chem. Soc., 90, 2074 (1968); R. Neher, B. Riniker, H. Zuber, W. Rittel and F. W. Kahnt, Helv. Chim. Acta, 51, 917 (1968).
39. H. Neher, B. Riniker, W. Rittel and H. Zuber, Helv. Chim. Acta, 51, 1900 (1968).

Chapter 25.
Relationship between Nucleoside Conformation and Biological Activity

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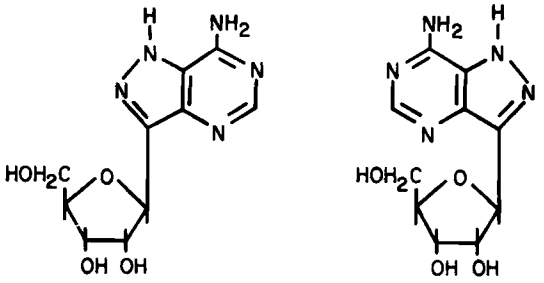
The aim of this review is to outline some of the effects of nucleoside conformation on the physical and biological properties of polynucleotides and on enzyme-substrate interactions. The following subjects are considered briefly: (a) some properties of synthetic and antibiotic nucleosides which help to establish the relationship between conformation and biological activity, (2) the relationship between substrate conformation and the specificity of two enzymes: pancreatic ribonuclease and adenosine deaminase, (3) the syn-anti conformational equilibrium as one possible determinant of polynucleotide helix stability, (4) the potential role of minor bases, such as pseudouridine, in maintaining the tertiary structure of tRNA and other polynucleotides.

Nucleoside and Polynucleotide Conformation - The conformation of nucleosides, nucleotides, and oligo- and polynucleotides has been studied extensively by means of X-ray diffraction and spectroscopic techniques, including nuclear magnetic resonance, optical rotatory dispersion and circular dichroism[1-3]. Such studies have shown that two major conformational elements can influence the three-dimensional structure of both nucleoside monomers and polymers: these are the relative orientations of the sugar and aglycones at the glycosyl bond[4] and the type of pucker in the sugar ring[5, 6]. In the case of polymers, the series of single bonds which form the phosphodiester linkages between adjacent nucleotides could, in principle, permit free rotation and therefore conformational modifications in the polynucleotide chain. However, the structure of the phosphodiester backbone is uniform in all known polynucleotides, and there is no evidence to implicate it as an important determinant of conformational variation.

When a carbon atom of the sugar ring is displaced (by about 0.5 Å) from the mean plane formed by the remaining four atoms, the ring is said to be puckered. Nearly all nucleosides whose structures have been determined crystallographically possess sugar puckers that are either C2' endo or C3' endo; that is, the respective carbons are located on the same side of the sugar plane as C5'. The data of Haschemeyer and Rich[5] indicate that the relative orientation of the nucleoside components at the glycosyl bond and therefore the conformation of the nucleoside are not influenced significantly by these different types of sugar pucker. Consequently, further discussion will be limited to properties associated with changes at the glycosyl bond.

The relative orientation of the sugar and base is defined in terms of a torsion angle ØCN, which refers to the angle formed between the plane of the base and the C1-O bond of the sugar, viewed along the glycosyl bond[4]. Examination of molecular models of nucleosides reveals that there are two ranges of torsional angles within which relatively stable conformations can be assumed. Each of these ranges, defined as the syn and anti conformation, covers somewhat more than 90°. The structure of formycin in the syn and anti conformation is illustrated in Fig. 1.



Formycin (anti) Formycin (syn)

Figure 1

Crystallographic analyses of nucleosides and their derivatives have revealed that the values of ØCN for the vast majority of these compounds correspond to the anti conformation[1,7]. The exceptions observed to date which possess the syn conformation are 3', 5'-cyclic AMP[8], 2'-deoxyguanosine[9] (when it is in a crystalline complex with 5-bromo-2'-deoxycytidine), the nucleoside antibiotic formycin[10], 8-bromoguanosine and 8-bromoadenosine[11]. Solution measurements, based on NMR, circular dichroism and optical rotatory dispersion are generally in agreement with the X-ray data, (see paper of Ts'o et al[2] and literature cited therein); however, studies[12] using Nuclear Overhauser NMR suggest that AMP may also prefer the syn conformation.

In nucleosides there are a number of steric barriers which hinder free rotation around the glycosyl bond and thus tend to "fix" a compound in its preferred conformation[5]. In pyrimidine nucleosides the barriers to rotation about the glycosyl bond are the steric interaction of the C6H and 2-keto oxygen with a C2' proton of the sugar. In purine nucleosides the interatomic contacts of the N3 and C8H of the base with the C2' proton and the furanose oxygen of the sugar provide the chief impediments to free rotation. The barriers to interconversion of the syn and anti conformation are considerably higher for pyrimidine than for purine nucleosides so rotational mobility is more likely in the latter compounds. Similarly, pyrimidine nucleoside residues in polynucleotides are

subjected to greater rotational restrictions than the purine nucleoside residues.

The nucleoside conformation in the usual double stranded DNA or RNA helices is restricted to anti since this conformation is indispensable for ordinary base pairing in structures of the Watson-Crick type[4, 13]. Indeed, recent analyses of X-ray data[1, 7] show that the ØCN angles of nucleosides in natural or synthetic helical polymers are virtually identical with those found in crystals of the free nucleosides. The individual residues in single stranded polynucleotides might be expected to possess considerably more rotational freedom than in comparable multi-stranded structures. Nevertheless, the available information, based predominantly on optical measurements, suggests that the nucleosides in the usual single-stranded polymers also retain the anti conformation [2, 14]. However, studies of polynucleotides containing cytotoxic nucleoside analogues have brought to light exceptional properties which tend to attribute some biochemical and biological phenomena to conformational abnormalities.

Conformation of Polynucleotides Containing Cytotoxic Nucleoside Analogues - The suspected relationship between biological activity and nucleoside conformation will be illustrated by reference to the behavior of the following nucleoside analogues: The antibiotics formycin[15], laurusin[16] and tubercidin[17], and the synthetic cytotoxic nucleosides 8-aza-adenosine[18] and 8-azaguanosine[19]. Formycin (Fig. 1) is a highly cytotoxic analogue of adenosine[20], in which the C8 and N9 of adenosine have been interchanged. The obvious similarity to adenosine suggests that formycin compounds should substitute for the corresponding adenosine nucleotides in enzymatic reactions. Indeed, with the exception of NAD synthetase, formycin nucleotides efficiently replace the corresponding adenosine counterparts with enzymes of intermediary metabolism, polynucleotide synthesis and tRNA synthesis and function[21-24]. However, there are several unexpected characteristics of formycin nucleotides which appear paradoxical, and all of these are related to the synthesis, structure or function of repeating sequences of formycin residues in polymers[21,23]For example, the efficient utilization of formycin nucleotides by RNA polymerase and polynucleotide phosphorylase is dependent on the concurrent polymerization of other nucleotides, i. e. synthesis of the homopolymer, poly F, proceeds poorly. In contrast to poly A, poly F fails to direct the in vitro synthesis of polylysine, yet random copolymers containing a mixture of formycin and normal nucleotides efficiently code for in vitro polypeptide synthesis and their coding properties are indistinguishable from the corresponding polymers that contain adenosine. A further anomaly of poly F concerns its abnormal susceptibility to degradation by nucleases. Thus, poly F is readily degraded by the

"pyrimidine-specific" enzyme pancreatic ribonuclease, but it is highly resistant to degradation by non-specific nucleases such as micrococcal nuclease, and the phosphodiesterases of spleen and rattlesnake venom. The converse is true for poly A. Indeed in mixed copolymers of formycin and adenosine the susceptibility to pancreatic RNase and the resistance to non-specific nucleases is directly related to the formycin content of the polymer.

The thermal denaturation profile, and the ultraviolet absorption and fluorescence spectra show that poly F is single-stranded at neutral pH. Consequently, the biochemical anomalies listed above reflect the properties of repeating sequences of formycin residues in the single-stranded state. The suspicion that such sequences exist in an abnormal conformation is substantiated by a number of observations[21]. Because the UV absorption maximum of formycin (295 mu) is remote from those of the normal nucleotides, it is possible to monitor selectively the optical transitions of formycin residues in mixed copolymers and in mixtures of homopolymer pairs. This useful property permits the demonstration of important differences in the ORD spectra of formycin polymers, compared with those of the normal adenosine counterparts: (a) the long wavelength Cotton bands in the ORD spectrum of single-stranded poly F are inverted compared with those of poly A; (b) the ORD spectrum of the double-stranded homopolymer pair poly rF. rU is qualitatively the same as that of the comparable poly rA. rU. However, on thermal denaturation of poly rF. rU the rotational band due to formycin residues undergoes a complete inversion with a change in sign. The ORD spectrum of formycin residues in denatured poly rF. rU thus becomes identical with that of single-stranded poly F. No such changes in ORD spectra occur on denaturation of poly rA. rU. These results demonstrate that a change in conformation of formycin residues accompanies the transition from double- to single- strandedness, whereas no comparable modification is observed in adenosine residues. (c) The ORD profiles of single-stranded copolymers containing formycin and any one of the normal nucleosides are variable with respect to the formycin rotation and depend upon both the nature of the adjacent base and the polymer base composition[21, 25]. (d) The observed changes in the ORD of formycin polymers does not result from alterations of the UV absorption spectrum of formycin residues - the latter remains constant in all polymers.

All the above facts suggest that formycin residues in polynucleotides can exist in different conformations, the exact distribution of these depending on the proportion of formycin residues, base sequence and strandedness of the structure. What is the nature of the conformational changes, and which portion of the formycin molecule mediates them? The most persuasive arguments are drawn from the structure of formycin

itself, and suggest that the conformational changes occur at the glycosyl bond.

The major structural difference between formycin and adenosine is at the glycosyl bond and this is of significance for several reasons. First, the increased length of the C-C glycosyl bond in formycin compared with the C-N bond in adenosine should facilitate rotation given a suitable driving force. The observation that formycin maintains the syn conformation in crystals[10] suggests that such a driving force exists. In addition, the C8H of adenosine, whose interaction with atoms of the furanose sugar ring provides one of the major rotational barriers for purine nucleosides[5], is absent in formycin and this change further encourages rotational freedom.

The preceding considerations, together with others drawn from the results of model building studies and from an analysis of the substrate specificity of pancreatic RNase (v. infra) are discussed more fully elsewhere[22]; all are consistent with the following formulations: in contrast to normal nucleotides (1) formycin residues in polynucleotides are subject to conformational transitions which take place at the glycosyl bond. (2) The formycin nucleotides are (a) anti in ordered double-stranded structures of the Watson-Crick type; (b) syn in neutral poly F and (c) probably a mixture of syn and anti in single stranded copolymers.

Many of the unusual properties of formycin polymers are reproduced by some other purine polynucleotide analogues. One such example is laurusin, a naturally occurring deamination product of formycin. Like formycin, laurusin nucleotides are polymerized inefficiently in the absence of other nucleotides , and the resulting homopolymer has an inverted ORD spectrum[25]; it is strongly resistant to degradation by nonspecific nucleases[25], but is rapidly attacked by pancreatic RNase[22]. These findings all point to the syn conformation for the individual residues in polylaurusin.

Although 8-azaguanosine and 8-azaadenosine possess the normal C-N glycosyl bond, they are free of the steric conformational restraints produced by the C8H-sugar interactions. Due to extreme difficulties in their enzymatic synthesis[25], the available quantities of 8-azapurine polymers have been insufficient to permit detailed physical characterization. On the basis of present data, it is clear that the incorporation of these analogues into polynucleotide results in the expected "abnormal" sensitivity to pancreatic RNase[25, 26], and aberrant low thermostability[25]. The findings so far are therefore entirely consistent with the expectation that these residues can also exist in the syn conformation.

The highly cytotoxic nucleoside antibiotic tubercidin likewise appears to adopt an abnormal conformation in single-stranded homopolymers[27]. However, present data are inadequate to permit a specific assignment of the syn conformation to the individual residues in single-stranded poly-tubercidin.

The strongest evidence that the proposed conformational abnormalities actually occur in the polynucleotide analogues considered here emerges from the stereochemical analysis of the catalytic specificity of pancreatic RNase.

Stereochemical Analysis of the Substrate Specificity of Pancreatic RNase - It is well established that pancreatic RNase behaves as a rigorously pyrimidine-specific enzyme with ordinary substrates. Nevertheless ribopolymers containing formycin, laurusin, 8-azaadenosine or 8-azaguanosine can be considered as "true" substrates for pancreatic RNase for the following reasons[22, 25]: their degradation is not attributable to a contaminating enzyme since they are depolymerized at low enzyme concentrations like the normal substrates poly C or poly U. Furthermore, the rate of degradation is not affected by prior heating of the enzyme solution (80° for 10 min) to destroy potential contaminants. In addition, the analogue polymers effectively compete with poly C and poly U for degradation by RNase. Moreover, RNase derivatives modified at His-119 and Lys-41 which are inactive in digesting poly U and poly C also do not depolymerize the purine polynucleotide analogues.

Although the preceding observations would suggest that the purine polynucleotide analogues should be viewed as orthodox substrates for RNase, their susceptibility to the enzyme differs in one important respect from that of pyrimidine polymers. The degradation of pyrimidine polymers by pancreatic RNase occurs by a two step process: rapid internal transphosphorylation to an intermediate nucleoside 2', 3'-monophosphate followed by a slow hydrolysis to the nucleoside 3'-monophosphate. Unlike normal pyrimidine substrates, the homopolymers of the purine analogues are converted exclusively to the corresponding 2', 3'-cyclic phosphates which are immune to subsequent enzymatic hydrolysis. The substrate properties of polymers such as poly F can therefore be used to differentiate the two activities of the enzyme, i. e. transphosphorylation and hydrolysis. We can now attempt to formulate an explanation for all these observations by reconciling the available X-ray date concerning the structure of ribonuclease with the stereochemistry of the substrate molecules.

Crystallographic and NMR studies of pancreatic RNase have shown that the pyrimidine nucleotide inhibitors, such as 2', (3')5-iodo UMP,

bind to the enzyme in the anti conformation[28, 29]. Under these conditions the N3-H, 2-keto and 4-keto groups of the pyrimidine base may form H-bonds respectively with the hydroxyls of threonine-45 and serine-123, and with the amide NH bond of threonine-45[28, 30]. Assuming that inhibitory pyrimidine nucleotides are bound at the active site of RNase, we can then compare pyrimidine polynucleotides and the purine analogue polymers in terms of reasonable stereochemical homologies. At first sight, 3'-CMP and 3'-UMP bear little resemblance to the 3'-monophosphates of formycin or the other purine analogue nucleosides. However, an examination of the three dimensional structure of the nucleotides demonstrates that, as far as the distribution of H-bonding groups is concerned, they can be considered H-bonding analogues or hybrids of both CMP and UMP[22]. In Fig. 2, 3'-FMP (syn) is compared with a hybrid pyrimidine structure containing the H-bonding donor groups of both CMP and UMP (anti). In this comparison the 3'-phosphoryl groups of the nucleotides have been superimposed. The C7-NH_2 group of formycin is close to the C4-NH_2 group of cytosine and the donor N3-H of formycin is sterically equivalent to the N3-H of uridine. It is important to note that

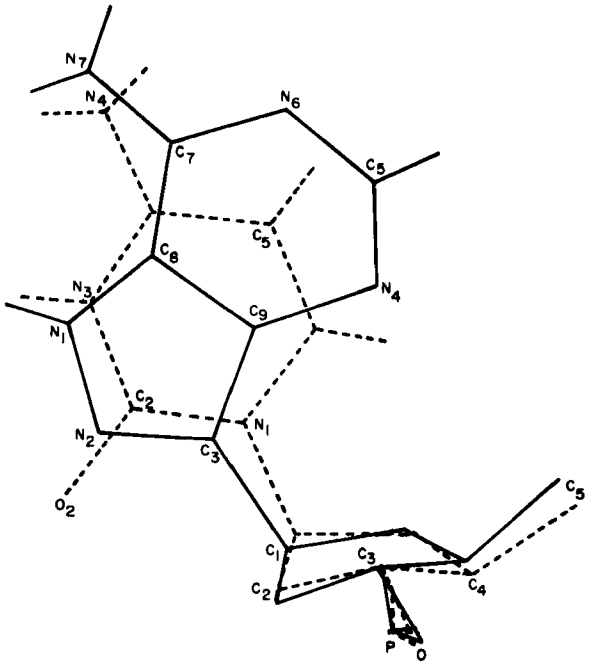


Fig. 2. Stereochemical comparison of 3'-FMP (syn) (solid line) and an H-bonding hybrid of 3'-CMP and 3'-UMP (anti) (dashed line). The torsion angles about the glycosyl bonds differ by 180° in the two nucleotides. All other torsion angles are identical and are like those in ribose nucleotides of helical RNA.

neither FMP nor any of the other purine analogue nucleotides possess a functional group homologous with the 2-keto group of the pyrimidines. We propose that formycin nucleotides (syn) interact with RNase through two H-bonds, in which both the C7-NH_2 and the N1-H of formycin act as donors. Using the same steric analogies laurusin (syn) can be seen to be an H-bonding analogue of uridine (anti) where the N1-H and N7-keto of laurusin are sterically equivalent to the N3-H and C4-keto of uridine. Similarly 8-azaguanosine (syn) provides two H-bond acceptor sites (N7 and C6-keto) whereas 8-azaadenosine (syn) provides one H-bond donor and one H-bond acceptor and it is thus an H-bonding analogue of cytidine (anti).

The importance of the conformational requirements outlined above can be illustrated by the stepwise degradation of the alternating copolymer poly r(F-U). When poly r(F-U) is exposed to RNase, it is first degraded to FpUp and then to 2', 3'-cyclic FMP and Up. In the two-stranded molecule formycin has the normal anti conformation. As such they are not acceptable substrates for RNase and the polymer is cleaved only at the uridine residues to yield FpUp. Once they are freed from the constraints imposed by the polymer structure, the formycin residues in these dinucleotides can assume the syn conformation, whereupon they are attacked by the enzyme.

The substrate specificity for binding and catalysis in the transphosphorylation reaction of RNase is now seen to be based on a system of stereochemically defined H-bonding groups. At least two of the relevant functional groups in the enzyme (the hydroxyls of serine and threonine) can act interchangeably as H-bond donors or acceptors. The complementary H-bonding groups in the substrate can be met by pyrimidine nucleotides in the anti conformation. The available H-bonding groups in purine nucleotide analogues can mimic those present in the pyrimidines, provided the purines are in the syn conformation. The fact that two H-bonding sites suffice for transphosphorylation does not exclude the possibility that three H-bonds may be formed normally by pyrimidine substrates or that two of the three H-bonds are sufficient for enzymatic activity. The observations do imply that the base-pairing region of the enzyme possesses functional groups capable of acting interchangeably as H-bond donors or acceptors. The threonine-45 and serine-123 hydroxyl groups are well suited for such a function.

As noted above, none of the purine analogue nucleotides (syn) present a structural homologue of the 2-keto group of the pyrimidine nucleotides (anti). There have been suggestions[31] that susceptibility to RNase was dependent on the presence of a keto group at a position α to the glycosyl bond. The efficient degradation of poly F and related structures demon-

strates that such a functional group is not required for transphosphorylation, although it cannot be excluded that a 2-keto group or its electronic equivalent act as important determinants for the hydrolysis reaction.

Effect of Nucleoside Conformation on Polynucleotide Helix Stability - As already noted, the complementarity observed in double-stranded structures of the Watson-Crick type requires the anti conformation in all residues which are involved in base pairing. The tendency of a significant proportion of H-bonded nucleotide units to adopt any conformation other than anti will produce a destabilizing effect which, under appropriate conditions, can be expressed as a decrease in thermostability (lowered T_m) of the helical structure. The thermal melting profiles of polymers containing formycin[21, 23], tubercidin[27], or 8-azaguanosine[32] substantiate these expectations. Thus, the thermal melting of the homopolymer pair poly rF. rU (T_m=22°), which is associated with the anti-syn interconversion of the formycin residues, occurs at a temperature much lower than that of the normal counterpart poly rA. rU (T_m=57°) under identical ionic conditions. Similarly, the corresponding homopolymer pair structure containing tubercidin (poly rTu. rU; T_m=31°) is much more thermolabile than poly rA. rU (T_m=57°); the melting of poly rTu. rU coincides with a conformational change in the tubercidin residues analogous to that observed for formycin polymers. The incorporation of 8-azaguanosine into tRNA[32] leads to a comparable decrease in thermostability; the T_m decreases progressively with increasing substitution of guanosine by 8-azaguanosine.

On the other hand, the tendency of formycin residues to assume the syn conformation can be markedly influenced by the rotational freedom of adjacent nucleotide residues. This can be illustrated by the properties of a family of alternating copolymers of the type poly r(F-U). In these polymers there is a perfectly alternating sequence of purine and pyrimidine residues and a pyrimidine is located on either side of each purine unit. Since the pyrimidine residues are restricted to the anti conformation, the rotational mobility of adjacent purines (e. g. formycin) is also limited. Thus the pyrimidines serve to maintain formycin residues in anti, and this can account for the fact that alternating copolymers of formycin show the same thermostability as the comparable adenosine copolymers. This is in contrast to the situation which exists in homopolymer pair structures, where all the formycin residues are in one strand and can undergo a conformational change in a cooperative manner.

Of the naturally occurring nucleosides, one representative - pseudouridine - is conspicuous as a potential mediator of conformational transitions resembling those produced by the purine nucleoside analogues. Like formycin and laurusin, pseudouridine is a C-glycosyl, and this can

be expected to lower markedly the energy barrier to rotation at the glycosyl bond. This is reflected in the fact that the alternating copolymer of formycin and pseudouridine, poly r(F-Ψ) shows an unexpectedly low T_m compared with other pseudouridine polymers[21]. The observation that cyanoethylation of pseudouridine residues in tRNA[33, 34] is associated with complete loss of amino acid acceptor activity and with physical evidence of modified tertiary structure is highly suggestive of an important role for this nucleoside in maintaining the functional architecture of tRNA.

General Considerations of Nucleoside Conformation - The interpretation proposed above for the susceptibility of poly F to degradation by RNase suggests that the specificity of the enzyme is dependent on its ability to interact with substrates through a properly oriented set of stereospecific H-bonding groups. However, there are numerous biological processes whose course is determined by molecular interactions based on stereospecific H-bonding, and the binding of substrates to RNase is only one example of this class. The annealing of single-stranded polynucleotides to form multistranded structures depends likewise on a system of H-bonds whose spatial distribution is restricted by the geometry of helical complexes; assuming that the appropriate H-bonding functions are present, their capacity to interact with complementary strands will be determined by a number of factors, one of which is the conformation of the individual nucleotide residues in the polymer. Another example of this type is the interaction between polynucleotide templates (messenger RNA's) and aminoacyl-tRNA. All available evidence suggests that codon-anti codon recognition is based on complementarity of the Watson-Crick type.[38] Since paired structures of this kind requires the anti conformation, it is not surprising that poly F, whose individual nucleotides are syn, fails to act as a template for polypeptide synthesis. Indeed, given the complementarity rules which are obeyed during replication, transcription and translation, it would be expected that the normal nucleotides found in polymers have been selected by nature precisely because they maintain the anti conformation under all conditions of polynucleotide function. That being so, some of the common nucleases which degrade ordinary polynucleotides could well have evolved to interact primarily with polynucleotides whose nucleoside components are anti. It is of interest, in this regard, that the diesterases from spleen and snake venom, and micrococcal nuclease, do not digest poly F, which is syn: also that the specificity of pancreatic RNase for the natural substrates - uridine and cytidine - is based on their normal anti conformation.

As illustrated by the crystallographic studies of Phillips et al[35, 36], stereospecific H-bonding is an important determinant in the binding of small molecule substrates by lysozyme. In the light of the work on

RNase and lysozyme, it would be expected that some of the interactions between monomeric nucleoside derivatives and enzymes of intermediary metabolism should be based on H-bonding. It is known that the substrate specificities of some of these enzymes depend on functional groups both in the sugar and the base[37]. Consequently, the relative orientation of these two rings, i. e. the nucleoside conformation at the glycosyl bond - should influence the substrate spectrum of such enzymes. The nucleoside analogues considered above are conformationally abnormal when polymerized, and the number of possible conformations is severaly restricted by the polymer structure even in the single-stranded state. However, as monomers these compounds, possessing virtually no barrier to free rotation, can be expected readily to adopt a conformation which will accommodate the H-bonding and steric requirements of an enzyme. Nucleosides restricted to one type of conformation, but retaining unmodified H-bonding groups, might be used to probe such conformational requirements. While the available data are insufficient to support definitive conclusions, the studies of Robins and co-workers[39] on adenosine deaminase are quite consistent with the view that nucleoside conformation is an important element in this substrate-enzyme interaction. These investigators examined a large series of 8-substituted adenosines and observed that most of the compounds did not interact with adenosine deaminase. Two of the compounds are slowly deaminated: 8-amino- and 8-ketoadenosine. The 8-substituents here are small enough to permit the syn-anti interconversion and the kinetics of this process could well be the rate-limiting step in catalysis if the enzyme is specific for the anti conformation. In the remaining compounds, larger substituents presumably restrict the conformation to syn at all times. Although this explanation is attractive, it should be emphasized that others cannot be excluded. For example, the inductive effect of many of the substituents might change the electronic structure of the purine ring and adversely influence substrate properties in this way. Nevertheless, for reasons which cannot be fully developed here, we tentatively favor the predominant role of substrate nucleoside conformation in this instance.

Present knowledge concerning the influence of nucleoside conformation in biological processes is too fragmentary to permit a reliable assessment of its general importance. In view of the ubiquity and central role of nucleotides in cellular function, the design, synthesis, and biochemical investigation of sterically restricted nucleosides might contribute a new set of biochemical probes. These could yield diverse insights of general metabolic significance.

References

1. S. Arnott and D. W. L. Hukins, Nature 244, 886 (1969).
2. P. O. P. Ts'o, N. S. Kondo, M. P. Schweizer and D. P. Hollis, Biochemistry 8, 997 (1969).
3. P. O. P. Ts'o, A. Rapaport and F. J. Bollum, Biochemistry 5, 4153 (1966).
4. J. Donohue and K. N. Trueblood, J. Mol. Biol. 2, 363 (1960).
5. A. E. V. Haschemeyer and A. Rich, J. Mol. Biol. 27, 369 (1967).
6. A. V. Lakshminarayanan and V. Sasisekharan, Biochim. Biophys. Acta, 204, 49 (1970).
7. M. Sundralingam, Biopolymers 7, 821 (1969).
8. K. Watenpaugh, J. Dow, L. H. Jensen and S. Furberg, Science 159, 206 (1968).
9. A. E. V. Haschemeyer and H. M. Sobel, Nature 202, 969 (1964).
10. G. Koyama, K. Maeda, H. Umezawa and Y. Iitaka, Tetrahedron Letters p. 597 (1966).
11. S. S. Tavale and H. M. Sobel, J. Mol. Biol. 48, 109 (1970).
12. P. A. Hart and J. P. Davis, J. Amer. Chem. Soc. 91, 512 (1969).
13. J. D. Watson and F. H. C. Crick, Nature 171, 373 (1953).
14. D. N. Holcomb and I. Tinoco, Jr. Biopolymers 3, 121 (1965).
15. R. K. Robins, L. B. Townsend, F. Cassidy, J. K. Gerster, A. F. Lewis and R. L. Miller, J. Heterocyclic Chem. 3, 110 (1966).
16. G. Koyama and H. Umezawa, J. Antibiot. (Tokyo) 18A, 175 (1965).
17. J. J. Fox, K. A. Watanabe and A. Bloch, in Progress in Nucleic Acid Research and Molecular Biology, Vol. 5, 251, Academic Press, New York (1966).
18. J. Baddiley, J. G. Buchanan and G. O. Osborne, J. Chem. Soc. 3606 (1958).
19. D. J. Hutchison, in Advances in Cancer Chemotherapy (A. Haddow and S. Weinhouse, Eds.) Vol. 7, p. 236, Academic Press, New York (1963).
20. H. Umezawa, T. Sawa, Y. Fukagawa, G. Koyama, M. Murase, M. Hamada and T. Takeuchi, J. Antibiot. (Tokyo), 18A, 178 (1965).
21. D. C. Ward and E. Reich, Proc. Nat. Acad. Sci. U.S.A. 61, 1494 (1968).
22. D. C. Ward, W. Fuller and E. Reich, Proc. Nat. Acad. Sci. U.S.A. 62, 581 (1969).
23. D. C. Ward, A. Cerami, E. Reich, G. Acs and L. Altwerger, J. Biol. Chem. 244, 3243 (1969).
24. D. C. Ward, E. Reich and L. Stryer, J. Biol. Chem. 244, 1228 (1969).
25. D. C. Ward and E. Reich, unpublished results.

26. D. H. Levin, Biochim. Biophys. Acta 61, 75 (1962).
27. D. C. Ward, A. Cerami, E. Reich, N. Mendelsohn and G. Acs. Submitted to J. Biol. Chem. 1970.
28. N. Allewell, Ph.D. Thesis, Yale University, New Haven, Connecticut (1969).
29. G. C. K. Roberts, E. A. Dennis, D. H. Meadows, J. S. Cohen and O. Jardetsky, Proc. Nat. Acad. Sci. U.S.A. 62, 1151 (1969).
30. F. M. Richards, H. W. Wyckoff and N. Allewell, in The Neurosciences: Second Study Program (F. O. Schmitt, editor-in-chief) The Rockefeller Press, New York, N.Y., to be published in 1970.
31. H. G. Gassen and H. Witzel, European J. Biochem. 1, 36 (1967).
32. D. H. Levin and M. Litt, J. Mol. Biol. 14, 506 (1965).
33. M. A. Siddigui, M. Krausopf and J. Ofengand, Biochim. Biophys. Res. Comm. 38, 156 (1970).
34. D. B. Miller, Biochim. Biophys. Acta, 174, 32 (1969).
35. C. C. F. Blake, L. N. Johnson, G. A. Mair, A. C. T. North, D. C. Phillips and V. R. Sarma, Proc. Roy. Soc. (London) B167, 378 (1967).
36. D. M. Chipman, V. Grisaro and N. Sharon, J. Biol. Chem. 242, 4388 (1967).
37. B. R. Baker, "Design of Active-Site Directed Irreversible Enzyme Inhibitors", J. Wiley and Sons Inc., New York, N.Y. (1967).
38. F. H. C. Crick, J. Mol. Biol. 19, 548 (1966).
39. L. W. Simon, R. J. Bauer, R. L. Tolman and R. K. Robins, Biochemistry 9, 573 (1970).

Section VI - Topics in Chemistry

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Chapter 26. Physicochemical Parameters in Drug Design

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Introduction - The literature on drug design shows an increase in the use of quantitative structure-activity relationships. Another area of interest in drug design that is growing rapidly is the use of molecular orbital theory in the direct correlation of electronic structure with biological response[1,2] and in the theoretical interpretation of empirically derived substituent constants.[3,4] For background, the reader is referred to a chapter by Purcell and Clayton[5] and an informative article by Lien on the use of substituent constants and regression analyses in structure-activity correlation studies.[6]

Quantitative Structure-Activity Relationships
Mathematical Model - The 1969 literature on quantitative structure-activity relationships contains much less about the mathematical (empirical, *de novo*) models than did the 1968 literature. To the authors' knowledge, no publications, other than those by Ban and Fujita[7] and Beasley and Purcell[8] which were cited in this chapter last year,[5] utilizing a purely mathematical, empirical correlation technique (such as that of Free and Wilson[9]) have appeared this year.

Linear Free-Energy-Related Model - On the other hand, more emphasis has been placed on the correlation of observed biological activity with measured physicochemical parameters of related compounds than in previous years. Hansch has given the historical development of the use of substituent constants and certain physicochemical parameters in quantitative structure-activity studies of biochemical systems.[10] Specifically, he has dealt with the dependence of biological activity of drug molecules upon partitioning properties, electronic parameters, and steric effects.[10]

Cammarata has presented an analysis of linear free-energy relationships which are postulated to exist in drug-

receptor interactions.[11] By separating the free-energy change occurring in a reaction into its electronic, desolvation, and steric components according to Leffler and Grunwald;[12] defining each component in terms of contributions made to it; and approximating these contributions with quantum mechanical parameters, Cammarata has arrived at a theoretical interpretation of substituent constants in the context of biological linear free-energy relationships.[11]

Clayton and Purcell[14] have reported a comparison of the predictive utility of the mathematical model of Free and Wilson[9] and the linear free-energy-related model of Hansch.[13] In the study of butyrylcholinesterase-inhibitory potencies of 6 members of a homologous series of 1-decyl-3-carbamoylpiperidines, the free-energy-related model gave better correlations of molecular structure with biochemical activity than did the mathematical model.[14]

Parameters Employed in Structure-Activity Correlations

Hydrophobic Parameters (Partitioning Properties) - When using partition coefficients to correlate molecular structure with biological activity, it is desirable for the properties of the partitioning phases to be as similar to the biological "partitioning system" as possible. Hansch *et al.* have used octanol and water as the two partitioning phases.[13] Although this may not be the ideal reference system, the excellent correlations between π (π = log P_X - log P_H where P_X is the partition coefficient of the substituted molecule and P_H is the partition coefficient of the parent, unsubstituted molecule) obtained from the octanol/water model and biological activity indicates that relatively little improvement in structure-activity correlations would probably be obtained by using another partitioning system.[15]

Lien has reported an example of another partitioning system, oleyl alcohol/water, however, which gave excellent correlations between log P and the fungitoxicity of some imides and their N-$SCCl_3$ compounds.[16] A parabolic equation of log P accounted for 91% of the variance in the toxicities of these compounds to spore germination of *Stemphylium sarcinaeforme* and of the imide N-$SCCl_3$ compound against *Erysiphe graminis* on oats. Log P also correlated to a high degree parabolically with the toxicity of the imide N-$SCCl_3$ compounds against *Neurospora crassa* and linearly with the activity of these compounds against *Alternaria tenuis*.[16]

Penniston *et al.* have presented a theoretical analysis of the penetration of drug molecules to their sites of action based upon a model which treats the membrane or macromolecule as a single phase of lipid character and ignores the nature of

its structure.[17] In the actual biological situation a drug molecule must interact many times with serum proteins and cross several membranes before reaching its site of action. Their theoretical treatment approximated this with many phases of alternating lipid and aqueous character. The derived model for passive permeation through biological tissues justifies the parabolic equations which have been used in an empirical sense to describe the relationship between the biological activity of a drug and its hydrophobic character.[17]

Electronic Parameters - Garrett *et al.*[18] have found that the bacteriostatic activity of a series of *meta*- and *para*-substituted N_1-phenylsulfanilamides on *E. coli* correlated well with modified Hammett substituent constants.[19] Also, there was no significant dependence of activity upon the relative lipophilicity of the compounds studied. The variation among the substituent groups would be expected to influence electronic parameters of the molecules since the different substituent groups have quite different electronic structures.[18] On the other hand, hydrophobic parameters such as the π-substituent constant of Hansch[13] are usually influenced most when the variation in the substituent groups manifests itself in changes in the length or structure of an alkyl chain or other lipophilic moiety.

Hansch *et al.* have used the homolytic substituent constants, E_R, of Yamamoto and Otsu[20] for correlations with antibacterial activity against *E. coli* for a series of chloramphenicol derivatives.[21] To determine the importance of electronic effects relative to hydrophobic properties, the authors performed regression analyses on the data of Garrett *et al.*[22] Using E_R derived from free-radical reactions and the hydrophobic parameter, π, of Hansch,[13] they obtained eq 1 and eq 2

$$\log A = (2.7 \pm 1.9)\, E_R + (0.93 \pm 0.37) \quad \overset{n}{8} \quad \overset{r}{0.820} \quad \overset{s}{0.243} \qquad (1)$$

$$\log A = (0.15 \pm 0.43)\, \pi + (1.29 \pm 0.41) \quad 8 \quad 0.317 \quad 0.403 \qquad (2)$$

where A is the activity of the drug, n is the number of data points, r is the correlation coefficient, and s is the standard deviation.[21] It is apparent that the electronic parameter has a higher degree of correlation with activity than does the hydrophobic parameter. The authors, however, mentioned that the linear combination of the two substituent parameters gives a much better correlation than that shown in eq 1 and eq 2 for either parameter. These and previous results have led Hansch *et al.* to hypothesize a free-radical mechanism of antibacterial action for chloramphenicol derivatives.[21]

Another electronic parameter used in the correlation of structure with biological activity is the empirical measure of electron density, pK_a. Brown and Kipp have resolved the overall basicity for series of 4'-alkyl-4-dimethylaminoazobenzenes and prime-dimethyl-4-dimethylaminoazobenzenes (Fig. 1) into pK_{am} and pK_{azo}, which represent the pK_a of the two basic centers, the amino and azo nitrogens, respectively.[23] The electron densities so determined were then compared with

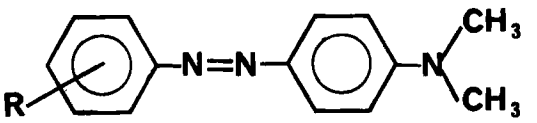

Figure 1. A prime-alkyl-4-dimethylaminoazobenzene

the relative hepatocarcinogenic activities. The results indicated a direct relationship between hepatocarcinogenic activity and the electron density at the amino nitrogen.[23]

Sasaki and Suzuki have compared partition coefficients and biological activities with the substituent constants σ_i and σ_π of Yukawa and Tsuno[24] which are useful in the estimation of π-electron charge density distributions.[25] They have suggested that both partition coefficient and biological activity are essentially dependent on the molecular electronic conditions.[25]

Steric Parameters - The steric parameter, E_s, of Taft[12] has been employed by Kutter and Hansch in the correlation of the molecular structure of some phenoxyethylcyclopropylamine monoamine oxidase inhibitors and diphenhydramine antihistamines with their activity.[26] For the types of biological activity investigated it was found that the Taft E_s was more important in the correlation than was either the electronic parameter of Hammett, σ,[27] or the hydrophobic parameter of Hansch.[13] Their results led Kutter and Hansch to conclude that the binding of substituent groups of moderate size into a macromolecular pouch may well be, at least over a limited range, a continuous linear process. This would negate the all-or-none situation which sometimes arises from the "lock and key" theory of enzyme-substrate interaction.[26]

Several substituent constants, including the Taft E_s,[12] have been employed by Hamor and Lien in the study of anticonvulsant activities of alkyl esters of 2-sulfamoylbenzoic acid against maximal electroshock and against strychnine-induced convulsions.[28] The steric substituent constant, E_s,[12] the electronic substituent constants, σ[27] and σ^*,[12] and log P calculated from Hansch's π values[13] were examined for correlation with biological response. For antistrychnine action, the best

correlation was obtained with an equation incorporating E_s, log P, and σ^*. Antielectroshock action correlated best with an equation incorporating E_s, log P, σ^*, and σ. The observation of a negative dependence on log P led Hamor and Lien[28] to suggest that these esters have sites of action which differ from those of barbiturates and other hypnotics since parabolic dependence on log P with an optimum value of about 2 has been reported for various hypnotics.[29]

Quantum Chemical Parameters - Rogers and Cammarata have attempted to correlate experimentally determined partition coefficients with molecular orbital indices by utilizing linear multiple regression analyses.[3] Their results indicated that the two molecular orbital indices, charge density, Q_r, and electrophilic superdelocalizability, S_r, may be related to the partitioning process. In explanation of the high degree of correlation between the experimental and calculated partition coefficients, Rogers and Cammarata have suggested that the magnitude of Q_r, a result of the π-electron delocalization of aromatic molecules, may reflect aqueous solubilization of the aromatic compound through a classical charge-dipole interaction mechanism. S_r, an index of the probability of formation of a weak π bond between an attacking reagent and a specific atom in the substrate during the progression of a chemical reaction, may be an indication of the extent of formation of a weak π bond between octanol and the substrate in octanol/water partition studies.[3]

In a more lengthy treatment, Rogers and Cammarata have suggested that σ charge calculations must be incorporated into the charge-density calculations in compounds which contain carboxy, hydroxy, amino, or other groups which can hydrogen bond with an aqueous solvent.[4] Since the formation of hydrogen bonds is greatly influenced by the σ charge on the electronegative atom, the use of π molecular orbital indices alone in these compounds ordinarily provides poor correlations with partition coefficients.[4]

Hermann *et al.* have calculated substituent constants for a series of substituted acetophenones based on several different molecular parameters and have compared these constants with the relative substrate efficiencies of the compounds toward a rabbit kidney reductase.[30] Substituent constants, E_c, analogous to Hammett σ values were calculated by the extended Hückel theory, EHT, of Hoffmann[31] and by the complete neglect of differential overlap, CNDO/2, treatment of Pople and Segal[32] which takes into account electron repulsions ignored in EHT.[30] The substituent constants were obtained by subtracting the electron density value for acetophenone from that for the derivative and multiplying by -1000. This permits

the sign of the calculated substituent constant to have the same significance as that of the Hammett σ. An analogous substituent constant, θ, was derived to "account for the immediate environment of the reactive center of the acetophenone derivative as seen by an approaching hydride ion," *i.e.*, the hydride ion interaction energy.[30] In addition, a substituent constant, δE, based on energy differences between ground and incipient transition states was derived by CNDO/2 treatment. Correlations obtained using the derived constants were not quite so good as the correlations obtained by using the experimentally derived substituent constants of Hansch.[30] It was noted, however, that direct molecular orbital calculations of various molecular properties allow one to examine the summation of these contributions at a specific position. On the other hand, experimentally derived substituent constants can only infer the summation of these contributions in terms of the rates of the specific reaction used to measure the substituent effect.[30]

Thermodynamic and Other Parameters - Ostrenga has illustrated the use of molar attraction constants in the correlation of molecular structure with biological activity.[33] The molar attraction constant F is regarded by Ostrenga as "a measure of the intermolecular attractive forces of a chemical species relative to a second entity." The mathematical definition of F is given by eq 3 where δ is a solubility parameter,[34] E is

$$\delta = (EV)^{1/2}/V = F/V \qquad (3)$$

the potential energy, and V is the molar volume. Thus, by obtaining values for δ and V, one can determine F for various compounds. By determining F for a substituted and an unsubstituted compound, one can determine the contribution of a substituent group to the molar attraction constant. By addition of previously determined substituent contributions to F,[35,36] Ostrenga calculated the expected biological activities of six different classes of compounds.[33] Where statistical comparisons were made, it was found that F correlated with biological activity at least as well as, and in some examples better than, the π value[13] of Hansch.[33]

In another study, Turner and Battershell have considered the relative influence of chemical reactivity, vapor pressure, and oil/water partition coefficients (obtained from R_m values[37]) on the fungicidal activity of a series of halogenated isophthalonitriles.[38] They obtained excellent correlations (r^2 = 0.96 and 0.94) between biological activity and the reactivity of the compounds with a model thiol compound and suggested that the mode of action involves reaction with thiol groups.[38]

Using multiple regression analyses, Jones *et al.* have correlated some physical organic parameters with the penetra-

tion and detoxication of some substituted phenyl N-methylcarbamates.[39] Although correlations using field constants, F,[40] and resonance parameters, R,[40] were also considered, cholinesterase (ChE) inhibition in terms of log $1/I_{50}$ (I_{50} = molar concentration of compound necessary to effect 50% inhibition) accounted for 50% of the variation in synergized LD_{50} values for the *meta*-substituted compounds.[39] In this example, liposolubility made no significant additional contribution to the correlation. On the other hand, with the *para*-substituted compounds both ChE inhibition and lipophilicity contributed significantly to the variation in the biological response. There was, however, no correlation of any parameters with the detoxication of these compounds.[39]

Similar studies by Fukuto *et al.* have correlated chemical reactivity, anti-ChE activity, and toxicity to insects of a series of oximes of substituted acetophenones and benzaldehydes with the free-energy parameters,[41] F,[40] R,[40] π,[13] and σ^*.[12] Excellent correlation was obtained between these electronic effects, reactivity, and anti-ChE activity (log $1/I_{50}$) for the ring-substituted acetophenone oximes studied. In the example of the benzaldehyde oximes, the addition of Hansch's π constant to the equation was necessary for correlation with the anti-ChE activity.[41]

Using a variety of physicochemical parameters, Kakeya *et al.* have performed a structure-activity study on a series of sulfonamide carbonic anhydrase inhibitors.[42] Hammett's σ constants were found to vary linearly with pK_a, chemical shift of the sulfamoyl protons, and the valence-force constant, f_r, of the S=O bond. It was found that compounds with a large σ value, a large f_r for the S=O bond, and a large chemical shift for the sulfamoyl group showed a strong inhibitory activity for carbonic anhydrase; this illustrates the importance of electronic effects in the activity of sulfonamide derivatives.[42]

Comparison of Parameters Used in Structure-Activity Studies - Leo *et al.* reported a comparison of parameters currently used in studies of structure-activity relationships.[15] The activities of a variety of molecular types, as measured in four different biological systems, were correlated with octanol/water partition coefficients,[13] polarizabilities,[43] molar attraction constants,[33] parachors,[44] adjusted parachors,[44] and molecular weights. In the systems investigated the octanol/water partition coefficient was found to correlate best with biological activity while adjusted parachor and molar attraction constant gave the next best correlations.[15]

Quantum Chemical Applications to Drug Design - Efforts to characterize the dynamic properties of biological systems by the application of molecular orbital theory have increased

substantially this year.[1,2,45-47] One example of this was given in a recent symposium on physicochemical mechanisms of carcinogenesis during which Pullman *et al.* presented an account of the present status of the application of quantum theory to chemical carcinogenesis.[48,49] They discussed the results of research on the nature of the carcinogenic interaction, the principal receptor, and the proximate carcinogen (*i.e.*, the initial molecule or metabolite that interacts directly with the receptor), and the relation of carcinogenesis to mutagenesis.[48]

In an effort to determine if there is a significant relationship between quantum mechanical parameters and therapeutic effectiveness, Sharpless and Greenblatt have studied a large series of acridines.[1] They have calculated the electron density, q, at the ring nitrogen, the energy of the highest occupied molecular orbital, HOMO, and the energy of the lowest empty molecular orbital, LEMO, for these compounds and have compared these parameters with their toxicities against three gram-positive microorganisms (*Clostridium welchii*, *Streptococcus pyogenes*, and *Staphylococcus aureus*) and two gram-negative microorganisms (*Bacterium coli* and *Proteus*). These quantum mechanical parameters were studied in terms of the pK_a values and molecular structures. Their results showed that q, LEMO, and pK_a correlated well with the toxicities of the acridines on the three gram-positive organisms but correlated poorly with their toxicities on gram-negative organisms.[1] They also found that the energy of the HOMO correlated poorly, if at all, with the therapeutic indices in all types of molecules and organisms investigated. This indicates that the toxicities of acridines should not be attributed to their electron-donating ability. The authors also proposed a membrane model that accounts for the fact that acridines are most effective therapeutically when in their most ionized state. Their model suggests that a positively charged molecule interacts with electrons at the cell surface; this "passport of neutrality" then allows it to cross the lipid phase of the membrane.[1]

Another example of the application of molecular orbital theory to drug design has been given by Andrews.[2] Molecular orbital calculations by the EHT and CNDO/2 methods were made on a number of anticonvulsant drugs and related compounds. His calculated dipole moments support the generalization that the CNDO/2 method is superior to the EHT method in assessing net atomic charges. These calculations indicate that the net charge at the "biologically active center" proposed by Perkow[50] does not determine the type or degree of central nervous-system activity of the drugs studied. They also show that hydrogen-bonding ability, in terms of net atomic charges, is unrelated to the type or extent of activity.[2]

Application of Nuclear Magnetic Resonance to Investigations of Drug-Receptor Interactions - The application of nuclear magnetic resonance spectroscopy to the study of interactions between small molecules and macromolecules has increased appreciably in the past five years. The technique of following the change effected in the relaxation rates of the protons of a small molecule by binding to a macromolecule has now been applied to the study of enzyme-substrate interactions,[51,52] enzyme-inhibitor interactions,[51,53] and enzyme-coenzyme interactions.[54] Recently, Fischer and Jost have directly observed the interaction of a drug with its receptor site in an intact cellular system by this method.[55] By observing the differential broadening of the peaks associated with the protons of epinephrine upon the addition of a single-cell suspension of mouse liver cells (Fig. 2), they were able to draw conclusions about the nature of the binding of epinephrine to its receptor site in the mouse liver cell. The marked broadening of the phenyl and methylene peaks of epinephrine indicates that the molecule was being bound both at the ring and somewhere distal to the methylene group on the side chain.[55] The broadening of the methyl peak was less noticeable and was attributed to the group's retaining considerable freedom of motion even when the complex is formed with the receptor site.[55]

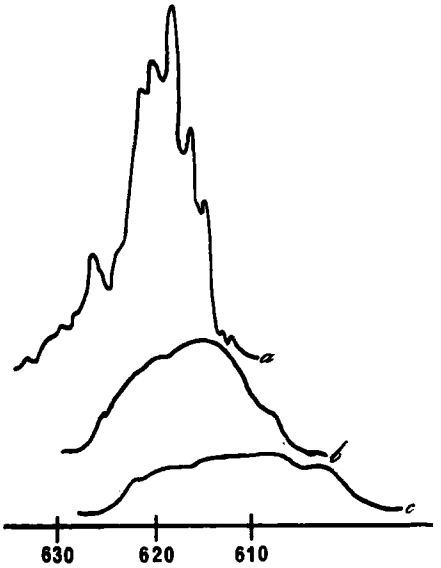

Figure 2. NMR signal arising from the phenyl protons of epinephrine.[55] Scale: chemical shift in Hertz. a. Epinephrine. b. Liver cells with 0.01M epinephrine. c. Liver cells with 0.005M epineprine.[55]

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References

1. N.E. Sharpless and C.L. Greenblatt, *Exp. Parasitol.*, 24, 216 (1969).
2. P.R. Andrews, *J. Med. Chem.*, 12, 761 (1969).
3. K.S. Rogers and A. Cammarata, *J. Med. Chem.*, 12, 692 (1969).
4. K.S. Rogers and A. Cammarata, *Biochim. Biophys. Acta*, 193, 22 (1969).

5. W.P. Purcell and J.M. Clayton, *Ann. Repts. Med. Chem., 1968*, 314 (1969).
6. E.J. Lien, *Amer. J. Pharm. Educ.*, 33, 368 (1969).
7. T. Ban and T. Fujita, *J. Med. Chem.*, 12, 353 (1969).
8. J.G. Beasley and W.P. Purcell, *Biochim. Biophys. Acta*, 178, 175 (1969).
9. S.M. Free, Jr. and J.W. Wilson, *J. Med. Chem.*, 7, 395 (1964).
10. C. Hansch, *Acc. Chem. Res.*, 2, 232 (1969).
11. A. Cammarata, *J. Med. Chem.*, 12, 314 (1969).
12. J.E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," John Wiley and Sons, Inc., New York, N.Y., 1963.
13. C. Hansch and T. Fujita, *J. Amer. Chem. Soc.*, 86, 1616 (1964).
14. J.M. Clayton and W.P. Purcell, *J. Med. Chem.*, 12, 1087 (1969).
15. A. Leo, C. Hansch, and C. Church, *J. Med. Chem.*, 12, 766 (1969).
16. E.J. Lien, *J. Agr. Food Chem.*, 17, 1265 (1969).
17. J.T. Penniston, L. Beckett, D.L. Bentley, and C. Hansch, *Mol. Pharmacol.*, 5, 333 (1969).
18. E.R. Garrett, J.B. Mielck, J.K. Seydel, and H.J. Kessler, *J. Med. Chem.*, 12, 740 (1969).
19. M. Yoshioka, K. Hamamoto, and T. Kubota, *Bull. Chem. Soc. Jap.*, 35, 1723 (1962).
20. T. Yamamoto and T. Otsu, *Chem. Ind.* (London), 787 (1967).
21. C. Hansch, E. Kutter, and A. Leo, *J. Med. Chem.*, 12, 746 (1969).
22. E.R. Garrett, O.K. Wright, G.H. Miller, and K.L. Smith, *J. Med. Chem.*, 9, 203 (1966).
23. E.V. Brown and W.H. Kipp, *Cancer Res.*, 29, 1341 (1969).
24. Y. Yukawa and Y. Tsuno, *J. Chem. Soc. Jap. (Pure Chemistry Section)*, 86, 873 (1965).
25. Y. Sasaki and M. Suzuki, *Chem. Pharm. Bull.* (Tokyo), 17, 1569 (1969).
26. E. Kutter and C. Hansch, *J. Med. Chem.*, 12, 647 (1969).
27. L.P. Hammett, *Chem. Rev.*, 17, 125 (1935).
28. G.H. Hamor and E.J. Lien, *Farmaco, Ed. Sci.*, 24, 704 (1969).
29. C.Hansch, A. Steward, S. Anderson, and D. Bentley, *J. Med. Chem.*, 11, 1 (1968).
30. R.B. Hermann, H.W. Culp, R.E. McMahon, and M.M. Marsh, *J. Med. Chem.*, 12, 749 (1969).
31. R. Hoffmann, *J. Chem. Phys.*, 39, 1397 (1963).
32. J.A. Pople and G.A. Segal, *J. Chem. Phys.*, 44, 3289 (1966).
33. J.A. Ostrenga, *J. Med. Chem.*, 12, 349 (1969).
34. D.D. Lawson and J.D. Ingham, *Nature*, 223, 614 (1969).
35. J. Brandrup and E.H. Immergut, "Polymer Handbook," Interscience Publishers, Inc., New York, N.Y., 1966, p.IV, 341.
36. P.A. Small, *J. Appl. Chem.* (London), 3, 71 (1953).

37. C.B.C. Boyce and B.V. Milborrow, *Nature*, 208, 537 (1965).
38. N.J. Turner and R.D. Battershell, *Contrib. Boyce Thompson Inst.*, 24, 139 (1969).
39. R.L. Jones, R.L. Metcalf, and T.R. Fukuto, *J. Econ. Entomol.*, 62, 801 (1969).
40. C.G. Swain and E.C. Lupton, Jr., *J. Amer. Chem. Soc.*, 90, 4328 (1968).
41. T.R. Fukuto, R.L. Metcalf, R.L. Jones, and R.O. Myers, *J. Agr. Food Chem.*, 17, 923 (1969).
42. N. Kakeya, M. Aoki, A. Kamada, and N. Yata, *Chem. Pharm. Bull.* (Tokyo), 17, 1010 (1969).
43. G.M. Barrow, "Physical Chemistry," 2nd ed., McGraw-Hill, New York, N.Y., 1966, p.432.
44. J.C. McGowan, *J. Appl. Chem.* (London), 4, 41 (1954).
45. W.P. Purcell and K. Sundaram, *J. Med. Chem.*, 12, 18 (1969).
46. W.B. Neeley, *J. Med. Chem.*, 12, 16 (1969).
47. J. Crow, O. Wassermann, and W.C. Holland, *J. Med. Chem.*, 12, 764 (1969).
48. A. Pullman and B. Pullman, "Physico-chemical Mechanisms of Carcinogenesis, The Jerusalem Symposia on Quantum Chemistry and Biochemistry, I," The Israel Academy of Sciences and Humanities, Jerusalem, 1969, p.9.
49. B. Pullman, A. Pullman, R. Umans, and B. Maigret, *ibid.*, p.325.
50. W. Perkow, *Arzneim.-Forsch.*, 10, 284 (1960).
51. P.G. Schmidt, G.R. Stark, and J.D. Baldeschwieler, *J. Biol. Chem.*, 244, 1860 (1969).
52. G. Kato, *Mol. Pharmacol.*, 5, 148 (1969).
53. M.A. Raftery, F.W. Dahlquist, S.M. Parsons, and R.G. Wolcott, *Proc. Nat. Acad. Sci. U.S.*, 62, 44 (1969).
54. D.P. Hollis, *Biochemistry*, 6, 2080 (1967).
55. J.J. Fischer and M.C. Jost, *Mol. Pharmacol.*, 5, 420 (1969).

Section VI - Topics in Chemistry

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Clayton and Purcell[14] have reported a comparison of the predictive utility of the mathematical model of Free and Wilson[9] and the linear free-energy-related model of Hansch.[13] In the study of butyrylcholinesterase-inhibitory potencies of 6 members of a homologous series of 1-decyl-3-carbamoylpiperidines, the free-energy-related model gave better correlations of molecular structure with biochemical activity than did the mathematical model.[14]

Parameters Employed in Structure-Activity Correlations

Hydrophobic Parameters (Partitioning Properties) - When using partition coefficients to correlate molecular structure with biological activity, it is desirable for the properties of the partitioning phases to be as similar to the biological "partitioning system" as possible. Hansch *et al.* have used octanol and water as the two partitioning phases.[13] Although this may not be the ideal reference system, the excellent correlations between π (π = log P_X - log P_H where P_X is the partition coefficient of the substituted molecule and P_H is the partition coefficient of the parent, unsubstituted molecule) obtained from the octanol/water model and biological activity indicates that relatively little improvement in structure-activity correlations would probably be obtained by using another partitioning system.[15]

Lien has reported an example of another partitioning system, oleyl alcohol/water, however, which gave excellent correlations between log P and the fungitoxicity of some imides and their N-$SCCl_3$ compounds.[16] A parabolic equation of log P accounted for 91% of the variance in the toxicities of these compounds to spore germination of *Stemphylium sarcinaeforme* and of the imide N-$SCCl_3$ compound against *Erysiphe graminis* on oats. Log P also correlated to a high degree parabolically with the toxicity of the imide N-$SCCl_3$ compounds against *Neurospora crassa* and linearly with the activity of these compounds against *Alternaria tenuis*.[16]

Penniston *et al.* have presented a theoretical analysis of the penetration of drug molecules to their sites of action based upon a model which treats the membrane or macromolecule as a single phase of lipid character and ignores the nature of

its structure.[17] In the actual biological situation a drug molecule must interact many times with serum proteins and cross several membranes before reaching its site of action. Their theoretical treatment approximated this with many phases of alternating lipid and aqueous character. The derived model for passive permeation through biological tissues justifies the parabolic equations which have been used in an empirical sense to describe the relationship between the biological activity of a drug and its hydrophobic character.[17]

Electronic Parameters - Garrett *et al.*[18] have found that the bacteriostatic activity of a series of *meta*- and *para*-substituted N_1-phenylsulfanilamides on *E. coli* correlated well with modified Hammett substituent constants.[19] Also, there was no significant dependence of activity upon the relative lipophilicity of the compounds studied. The variation among the substituent groups would be expected to influence electronic parameters of the molecules since the different substituent groups have quite different electronic structures.[18] On the other hand, hydrophobic parameters such as the π-substituent constant of Hansch[13] are usually influenced most when the variation in the substituent groups manifests itself in changes in the length or structure of an alkyl chain or other lipophilic moiety.

Hansch *et al.* have used the homolytic substituent constants, E_R, of Yamamoto and Otsu[20] for correlations with antibacterial activity against *E. coli* for a series of chloramphenicol derivatives.[21] To determine the importance of electronic effects relative to hydrophobic properties, the authors performed regression analyses on the data of Garrett *et al.*[22] Using E_R derived from free-radical reactions and the hydrophobic parameter, π, of Hansch,[13] they obtained eq 1 and eq 2

$$\log A = (2.7 \pm 1.9)\, E_R + (0.93 \pm 0.37) \quad \overset{n}{8} \quad \overset{r}{0.820} \quad \overset{s}{0.243} \qquad (1)$$

$$\log A = (0.15 \pm 0.43)\, \pi + (1.29 \pm 0.41) \quad 8 \quad 0.317 \quad 0.403 \qquad (2)$$

where A is the activity of the drug, n is the number of data points, r is the correlation coefficient, and s is the standard deviation.[21] It is apparent that the electronic parameter has a higher degree of correlation with activity than does the hydrophobic parameter. The authors, however, mentioned that the linear combination of the two substituent parameters gives a much better correlation than that shown in eq 1 and eq 2 for either parameter. These and previous results have led Hansch *et al.* to hypothesize a free-radical mechanism of antibacterial action for chloramphenicol derivatives.[21]

Another electronic parameter used in the correlation of structure with biological activity is the empirical measure of electron density, pK_a. Brown and Kipp have resolved the overall basicity for series of 4'-alkyl-4-dimethylaminoazobenzenes and prime-dimethyl-4-dimethylaminoazobenzenes (Fig. 1) into pK_{am} and pK_{azo}, which represent the pK_a of the two basic centers, the amino and azo nitrogens, respectively.[23] The electron densities so determined were then compared with

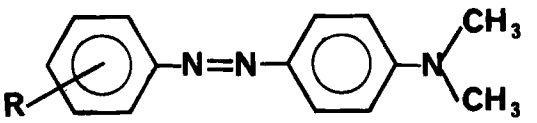

Figure 1. A prime-alkyl-4-dimethylaminoazobenzene

the relative hepatocarcinogenic activities. The results indicated a direct relationship between hepatocarcinogenic activity and the electron density at the amino nitrogen.[23]

Sasaki and Suzuki have compared partition coefficients and biological activities with the substituent constants σ_i and σ_π of Yukawa and Tsuno[24] which are useful in the estimation of π-electron charge density distributions.[25] They have suggested that both partition coefficient and biological activity are essentially dependent on the molecular electronic conditions.[25]

Steric Parameters - The steric parameter, E_s, of Taft[12] has been employed by Kutter and Hansch in the correlation of the molecular structure of some phenoxyethylcyclopropylamine monoamine oxidase inhibitors and diphenhydramine antihistamines with their activity.[26] For the types of biological activity investigated it was found that the Taft E_s was more important in the correlation than was either the electronic parameter of Hammett, σ,[27] or the hydrophobic parameter of Hansch.[13] Their results led Kutter and Hansch to conclude that the binding of substituent groups of moderate size into a macromolecular pouch may well be, at least over a limited range, a continuous linear process. This would negate the all-or-none situation which sometimes arises from the "lock and key" theory of enzyme-substrate interaction.[26]

Several substituent constants, including the Taft E_s,[12] have been employed by Hamor and Lien in the study of anticonvulsant activities of alkyl esters of 2-sulfamoylbenzoic acid against maximal electroshock and against strychnine-induced convulsions.[28] The steric substituent constant, E_s,[12] the electronic substituent constants, σ[27] and σ^*,[12] and log P calculated from Hansch's π values[13] were examined for correlation with biological response. For antistrychnine action, the best

correlation was obtained with an equation incorporating E_s, log P, and σ^*. Antielectroshock action correlated best with an equation incorporating E_s, log P, σ^*, and σ. The observation of a negative dependence on log P led Hamor and Lien[28] to suggest that these esters have sites of action which differ from those of barbiturates and other hypnotics since parabolic dependence on log P with an optimum value of about 2 has been reported for various hypnotics.[29]

Quantum Chemical Parameters - Rogers and Cammarata have attempted to correlate experimentally determined partition coefficients with molecular orbital indices by utilizing linear multiple regression analyses.[3] Their results indicated that the two molecular orbital indices, charge density, Q_r, and electrophilic superdelocalizability, S_r, may be related to the partitioning process. In explanation of the high degree of correlation between the experimental and calculated partition coefficients, Rogers and Cammarata have suggested that the magnitude of Q_r, a result of the π-electron delocalization of aromatic molecules, may reflect aqueous solubilization of the aromatic compound through a classical charge-dipole interaction mechanism. S_r, an index of the probability of formation of a weak π bond between an attacking reagent and a specific atom in the substrate during the progression of a chemical reaction, may be an indication of the extent of formation of a weak π bond between octanol and the substrate in octanol/water partition studies.[3]

In a more lengthy treatment, Rogers and Cammarata have suggested that σ charge calculations must be incorporated into the charge-density calculations in compounds which contain carboxy, hydroxy, amino, or other groups which can hydrogen bond with an aqueous solvent.[4] Since the formation of hydrogen bonds is greatly influenced by the σ charge on the electronegative atom, the use of π molecular orbital indices alone in these compounds ordinarily provides poor correlations with partition coefficients.[4]

Hermann *et al.* have calculated substituent constants for a series of substituted acetophenones based on several different molecular parameters and have compared these constants with the relative substrate efficiencies of the compounds toward a rabbit kidney reductase.[30] Substituent constants, E_c, analogous to Hammett σ values were calculated by the extended Hückel theory, EHT, of Hoffmann[31] and by the complete neglect of differential overlap, CNDO/2, treatment of Pople and Segal[32] which takes into account electron repulsions ignored in EHT.[30] The substituent constants were obtained by subtracting the electron density value for acetophenone from that for the derivative and multiplying by -1000. This permits

the sign of the calculated substituent constant to have the same significance as that of the Hammett σ. An analogous substituent constant, θ, was derived to "account for the immediate environment of the reactive center of the acetophenone derivative as seen by an approaching hydride ion," *i.e.*, the hydride ion interaction energy.[30] In addition, a substituent constant, δE, based on energy differences between ground and incipient transition states was derived by CNDO/2 treatment. Correlations obtained using the derived constants were not quite so good as the correlations obtained by using the experimentally derived substituent constants of Hansch.[30] It was noted, however, that direct molecular orbital calculations of various molecular properties allow one to examine the summation of these contributions at a specific position. On the other hand, experimentally derived substituent constants can only infer the summation of these contributions in terms of the rates of the specific reaction used to measure the substituent effect.[30]

Thermodynamic and Other Parameters - Ostrenga has illustrated the use of molar attraction constants in the correlation of molecular structure with biological activity.[33] The molar attraction constant F is regarded by Ostrenga as "a measure of the intermolecular attractive forces of a chemical species relative to a second entity." The mathematical definition of F is given by eq 3 where δ is a solubility parameter,[34] E is

$$\delta = (EV)^{1/2}/V = F/V \qquad (3)$$

the potential energy, and V is the molar volume. Thus, by obtaining values for δ and V, one can determine F for various compounds. By determining F for a substituted and an unsubstituted compound, one can determine the contribution of a substituent group to the molar attraction constant. By addition of previously determined substituent contributions to F,[35,36] Ostrenga calculated the expected biological activities of six different classes of compounds.[33] Where statistical comparisons were made, it was found that F correlated with biological activity at least as well as, and in some examples better than, the π value[13] of Hansch.[33]

In another study, Turner and Battershell have considered the relative influence of chemical reactivity, vapor pressure, and oil/water partition coefficients (obtained from R_m values[37]) on the fungicidal activity of a series of halogenated isophthalonitriles.[38] They obtained excellent correlations (r^2 = 0.96 and 0.94) between biological activity and the reactivity of the compounds with a model thiol compound and suggested that the mode of action involves reaction with thiol groups.[38]

Using multiple regression analyses, Jones *et al.* have correlated some physical organic parameters with the penetra-

tion and detoxication of some substituted phenyl N-methylcarbamates.[39] Although correlations using field constants, F,[40] and resonance parameters, R,[40] were also considered, cholinesterase (ChE) inhibition in terms of log $1/I_{50}$ (I_{50} = molar concentration of compound necessary to effect 50% inhibition) accounted for 50% of the variation in synergized LD_{50} values for the *meta*-substituted compounds.[39] In this example, liposolubility made no significant additional contribution to the correlation. On the other hand, with the *para*-substituted compounds both ChE inhibition and lipophilicity contributed significantly to the variation in the biological response. There was, however, no correlation of any parameters with the detoxication of these compounds.[39]

Similar studies by Fukuto *et al.* have correlated chemical reactivity, anti-ChE activity, and toxicity to insects of a series of oximes of substituted acetophenones and benzaldehydes with the free-energy parameters,[41] F,[40] R,[40] π,[13] and σ^*.[12] Excellent correlation was obtained between these electronic effects, reactivity, and anti-ChE activity (log $1/I_{50}$) for the ring-substituted acetophenone oximes studied. In the example of the benzaldehyde oximes, the addition of Hansch's π constant to the equation was necessary for correlation with the anti-ChE activity.[41]

Using a variety of physicochemical parameters, Kakeya *et al.* have performed a structure-activity study on a series of sulfonamide carbonic anhydrase inhibitors.[42] Hammett's σ constants were found to vary linearly with pK_a, chemical shift of the sulfamoyl protons, and the valence-force constant, f_r, of the S=O bond. It was found that compounds with a large σ value, a large f_r for the S=O bond, and a large chemical shift for the sulfamoyl group showed a strong inhibitory activity for carbonic anhydrase; this illustrates the importance of electronic effects in the activity of sulfonamide derivatives.[42]

Comparison of Parameters Used in Structure-Activity Studies - Leo *et al.* reported a comparison of parameters currently used in studies of structure-activity relationships.[15] The activities of a variety of molecular types, as measured in four different biological systems, were correlated with octanol/water partition coefficients,[13] polarizabilities,[43] molar attraction constants,[33] parachors,[44] adjusted parachors,[44] and molecular weights. In the systems investigated the octanol/water partition coefficient was found to correlate best with biological activity while adjusted parachor and molar attraction constant gave the next best correlations.[15]

Quantum Chemical Applications to Drug Design - Efforts to characterize the dynamic properties of biological systems by the application of molecular orbital theory have increased

substantially this year.[1,2,45-47] One example of this was given in a recent symposium on physicochemical mechanisms of carcinogenesis during which Pullman *et al.* presented an account of the present status of the application of quantum theory to chemical carcinogenesis.[48,49] They discussed the results of research on the nature of the carcinogenic interaction, the principal receptor, and the proximate carcinogen (*i.e.*, the initial molecule or metabolite that interacts directly with the receptor), and the relation of carcinogenesis to mutagenesis.[48]

In an effort to determine if there is a significant relationship between quantum mechanical parameters and therapeutic effectiveness, Sharpless and Greenblatt have studied a large series of acridines.[1] They have calculated the electron density, q, at the ring nitrogen, the energy of the highest occupied molecular orbital, HOMO, and the energy of the lowest empty molecular orbital, LEMO, for these compounds and have compared these parameters with their toxicities against three gram-positive microorganisms (*Clostridium welchii*, *Streptococcus pyogenes*, and *Staphylococcus aureus*) and two gram-negative microorganisms (*Bacterium coli* and *Proteus*). These quantum mechanical parameters were studied in terms of the pK_a values and molecular structures. Their results showed that q, LEMO, and pK_a correlated well with the toxicities of the acridines on the three gram-positive organisms but correlated poorly with their toxicities on gram-negative organisms.[1] They also found that the energy of the HOMO correlated poorly, if at all, with the therapeutic indices in all types of molecules and organisms investigated. This indicates that the toxicities of acridines should not be attributed to their electron-donating ability. The authors also proposed a membrane model that accounts for the fact that acridines are most effective therapeutically when in their most ionized state. Their model suggests that a positively charged molecule interacts with electrons at the cell surface; this "passport of neutrality" then allows it to cross the lipid phase of the membrane.[1]

Another example of the application of molecular orbital theory to drug design has been given by Andrews.[2] Molecular orbital calculations by the EHT and CNDO/2 methods were made on a number of anticonvulsant drugs and related compounds. His calculated dipole moments support the generalization that the CNDO/2 method is superior to the EHT method in assessing net atomic charges. These calculations indicate that the net charge at the "biologically active center" proposed by Perkow[50] does not determine the type or degree of central nervous-system activity of the drugs studied. They also show that hydrogen-bonding ability, in terms of net atomic charges, is unrelated to the type or extent of activity.[2]

Application of Nuclear Magnetic Resonance to Investigations of Drug-Receptor Interactions - The application of nuclear magnetic resonance spectroscopy to the study of interactions between small molecules and macromolecules has increased appreciably in the past five years. The technique of following the change effected in the relaxation rates of the protons of a small molecule by binding to a macromolecule has now been applied to the study of enzyme-substrate interactions,[51,52] enzyme-inhibitor interactions,[51,53] and enzyme-coenzyme interactions.[54] Recently, Fischer and Jost have directly observed the interaction of a drug with its receptor site in an intact cellular system by this method.[55] By observing the differential broadening of the peaks associated with the protons of epinephrine upon the addition of a single-cell suspension of mouse liver cells (Fig. 2), they were able to draw conclusions about the nature of the binding of epinephrine to its receptor site in the mouse liver cell. The marked broadening of the phenyl and methylene peaks of epinephrine indicates that the molecule was being bound both at the ring and somewhere distal to the methylene group on the side chain.[55] The broadening of the methyl peak was less noticeable and was attributed to the group's retaining considerable freedom of motion even when the complex is formed with the receptor site.[55]

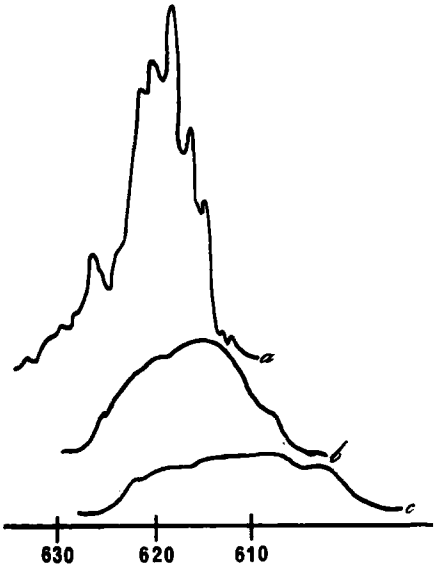

Figure 2. NMR signal arising from the phenyl protons of epinephrine.[55] Scale: chemical shift in Hertz. a. Epinephrine. b. Liver cells with 0.01M epinephrine. c. Liver cells with 0.005M epineprine.[55]

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References

1. N.E. Sharpless and C.L. Greenblatt, *Exp. Parasitol.*, 24, 216 (1969).
2. P.R. Andrews, *J. Med. Chem.*, 12, 761 (1969).
3. K.S. Rogers and A. Cammarata, *J. Med. Chem.*, 12, 692 (1969).
4. K.S. Rogers and A. Cammarata, *Biochim. Biophys. Acta*, 193, 22 (1969).

5. W.P. Purcell and J.M. Clayton, *Ann. Repts. Med. Chem., 1968*, 314 (1969).
6. E.J. Lien, *Amer. J. Pharm. Educ.*, 33, 368 (1969).
7. T. Ban and T. Fujita, *J. Med. Chem.*, 12, 353 (1969).
8. J.G. Beasley and W.P. Purcell, *Biochim. Biophys. Acta*, 178, 175 (1969).
9. S.M. Free, Jr. and J.W. Wilson, *J. Med. Chem.*, 7, 395 (1964).
10. C. Hansch, *Acc. Chem. Res.*, 2, 232 (1969).
11. A. Cammarata, *J. Med. Chem.*, 12, 314 (1969).
12. J.E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," John Wiley and Sons, Inc., New York, N.Y., 1963.
13. C. Hansch and T. Fujita, *J. Amer. Chem. Soc.*, 86, 1616 (1964).
14. J.M. Clayton and W.P. Purcell, *J. Med. Chem.*, 12, 1087 (1969).
15. A. Leo, C. Hansch, and C. Church, *J. Med. Chem.*, 12, 766 (1969).
16. E.J. Lien, *J. Agr. Food Chem.*, 17, 1265 (1969).
17. J.T. Penniston, L. Beckett, D.L. Bentley, and C. Hansch, *Mol. Pharmacol.*, 5, 333 (1969).
18. E.R. Garrett, J.B. Mielck, J.K. Seydel, and H.J. Kessler, *J. Med. Chem.*, 12, 740 (1969).
19. M. Yoshioka, K. Hamamoto, and T. Kubota, *Bull. Chem. Soc. Jap.*, 35, 1723 (1962).
20. T. Yamamoto and T. Otsu, *Chem. Ind.* (London), 787 (1967).
21. C. Hansch, E. Kutter, and A. Leo, *J. Med. Chem.*, 12, 746 (1969).
22. E.R. Garrett, O.K. Wright, G.H. Miller, and K.L. Smith, *J. Med. Chem.*, 9, 203 (1966).
23. E.V. Brown and W.H. Kipp, *Cancer Res.*, 29, 1341 (1969).
24. Y. Yukawa and Y. Tsuno, *J. Chem. Soc. Jap. (Pure Chemistry Section)*, 86, 873 (1965).
25. Y. Sasaki and M. Suzuki, *Chem. Pharm. Bull.* (Tokyo), 17, 1569 (1969).
26. E. Kutter and C. Hansch, *J. Med. Chem.*, 12, 647 (1969).
27. L.P. Hammett, *Chem. Rev.*, 17, 125 (1935).
28. G.H. Hamor and E.J. Lien, *Farmaco, Ed. Sci.*, 24, 704 (1969).
29. C.Hansch, A. Steward, S. Anderson, and D. Bentley, *J. Med. Chem.*, 11, 1 (1968).
30. R.B. Hermann, H.W. Culp, R.E. McMahon, and M.M. Marsh, *J. Med. Chem.*, 12, 749 (1969).
31. R. Hoffmann, *J. Chem. Phys.*, 39, 1397 (1963).
32. J.A. Pople and G.A. Segal, *J. Chem. Phys.*, 44, 3289 (1966).
33. J.A. Ostrenga, *J. Med. Chem.*, 12, 349 (1969).
34. D.D. Lawson and J.D. Ingham, *Nature*, 223, 614 (1969).
35. J. Brandrup and E.H. Immergut, "Polymer Handbook," Interscience Publishers, Inc., New York, N.Y., 1966, p.IV, 341.
36. P.A. Small, *J. Appl. Chem.* (London), 3, 71 (1953).

37. C.B.C. Boyce and B.V. Milborrow, *Nature*, 208, 537 (1965).
38. N.J. Turner and R.D. Battershell, *Contrib. Boyce Thompson Inst.*, 24, 139 (1969).
39. R.L. Jones, R.L. Metcalf, and T.R. Fukuto, *J. Econ. Entomol.*, 62, 801 (1969).
40. C.G. Swain and E.C. Lupton, Jr., *J. Amer. Chem. Soc.*, 90, 4328 (1968).
41. T.R. Fukuto, R.L. Metcalf, R.L. Jones, and R.O. Myers, *J. Agr. Food Chem.*, 17, 923 (1969).
42. N. Kakeya, M. Aoki, A. Kamada, and N. Yata, *Chem. Pharm. Bull.* (Tokyo), 17, 1010 (1969).
43. G.M. Barrow, "Physical Chemistry," 2nd ed., McGraw-Hill, New York, N.Y., 1966, p.432.
44. J.C. McGowan, *J. Appl. Chem.* (London), 4, 41 (1954).
45. W.P. Purcell and K. Sundaram, *J. Med. Chem.*, 12, 18 (1969).
46. W.B. Neeley, *J. Med. Chem.*, 12, 16 (1969).
47. J. Crow, O. Wassermann, and W.C. Holland, *J. Med. Chem.*, 12, 764 (1969).
48. A. Pullman and B. Pullman, "Physico-chemical Mechanisms of Carcinogenesis, The Jerusalem Symposia on Quantum Chemistry and Biochemistry, I," The Israel Academy of Sciences and Humanities, Jerusalem, 1969, p.9.
49. B. Pullman, A. Pullman, R. Umans, and B. Maigret, *ibid.*, p.325.
50. W. Perkow, *Arzneim.-Forsch.*, 10, 284 (1960).
51. P.G. Schmidt, G.R. Stark, and J.D. Baldeschwieler, *J. Biol. Chem.*, 244, 1860 (1969).
52. G. Kato, *Mol. Pharmacol.*, 5, 148 (1969).
53. M.A. Raftery, F.W. Dahlquist, S.M. Parsons, and R.G. Wolcott, *Proc. Nat. Acad. Sci. U.S.*, 62, 44 (1969).
54. D.P. Hollis, *Biochemistry*, 6, 2080 (1967).
55. J.J. Fischer and M.C. Jost, *Mol. Pharmacol.*, 5, 420 (1969).

Chapter 28. Recent Methods in Peptide Synthesis

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Introduction - This review is based primarily on papers published in 1969, and it is selective for the sake of brevity. It is limited to purely organic synthetic methods; structure-activity relationships are discussed in other chapters.

Books and Review Articles - A number of book and review articles have appeared during the last year. Advances in the chemical synthesis of peptides were reviewed by Hardy[1], Katsoyannis[2] and Young[3]. The description of the experimental details for the Merrifield method of peptide synthesis has been published by Stewart[4]. It is also noted that the tentative rules[5] for the abbreviated nomenclature of synthetic peptides have also been published.

Protecting Groups - A number of new protecting groups, some of which are variations of those already in use, have been introduced. Further work has appeared on the very interesting aralkyloxycarbonylamino protecting groups, with the synthesis of 2-(p-biphenylyl)isopropoxycarbonyl amino acids and their activated esters[6]. The N-protecting group was introduced by the use of 2-(p-biphenylyl)isopropylphenyl carbonate, a reagent which is stable at 0°. The advantage of this group is its ready removal by dilute acetic acid even in the presence of the t-butyloxycarbonyl (BOC) group. The useful derivatives, BOC-γ-benzyl-glutamic acid and BOC-β-benzyl-aspartic acid, have been prepared by an improved method[7]; also various S-substituted derivatives of cysteine have been converted to their corresponding BOC compounds[8] using the Schnabel method. An ion exchange method for selectively removing the BOC group, even in the presence of the tert-butyl ester, has been reported[9]. Other N-protecting groups which have received attention this year are the tert-amyloxycarbonyl[10], prepared from the corresponding chloroformate, and p-methoxybenzyloxycarbonyl, prepared either from its chloroformate or the 2, 4, 5-trichlorophenylcarbonate[11-12]. Both groups can be deblocked under mild conditions. The use of the p-methoxybenzyloxy-carbonyl protecting group has been illustrated in a new synthesis of oxytocin[13]. A new S-protecting group for cysteine, the β,β-diethoxycarbonylethyl residue has been suggested[14] and its use has been illustrated in a new synthesis of glutathione[15]. An improved synthesis of N(im)-benzylhistidine has been reported[16], without the use of sodium in liquid ammonia. The newer method of protecting the

imidazole ring of histidine with the dinitrophenyl group has been utilized in the synthesis of sequences of human hemoglobin β chain[17] using the solid phase technique for peptide synthesis. Also, the 2,2,2-trifluoro-1-(benzyloxycarbonylamino)ethyl group[18] has been used as a method of protecting the hydroxy functions of serine and threonine.

A new amino acid carboxyl protecting group, the 4-(methylthio)phenyl ester, has been developed[19]. These esters are easily prepared by the N,N'-dicyclohexylcarbodiimide method, and the BOC and carbobenzoxy protecting groups can be easily removed in their presence. The attractive feature of this protective ester is its facile conversion, by oxidation and without racemization[20], to the activated 4-(methylsulfonyl)phenyl ester. This method has been used successfully for peptide[21], polypeptide[22] and O-depsipeptide synthesis[23]. The preparation and properties of a number of 4-(methylthio)phenyl esters of amino acids have been reported[24], including new oxidation conditions which permit the method of protection and then activation to be applicable to nearly all of the usual protecting groups and the commonly occurring amino acids, with the exception of those containing sulfur. The preparation and properties of some 4-picolyl esters of amino acids have been reported [25]. These esters are cleaved by cold alkali, by catalytic hydrogenation, by sodium in liquid ammonia and by electrolytic reduction. A new procedure for the facilitation of peptide synthesis is also reported in which the carboxyl-terminal residue is incorporated as its 4-picolyl ester; after each coupling reaction the product is separated by extraction into an acidic phase.

Peptide Bond Formation - Most methods described for peptide formation have been dependent upon enhancement of the reactivity of one of the functional groups of the amino acid. Usually the carboxyl group of the amino acid is activated and, where possible, the activation of peptide terminal carboxyl groups is avoided because of dangers of racemization. Halogenated phenyl esters, especially the trichloro[26] and pentachlorophenyl esters[27-30], have received continuing attention. With the latter activated ester, no racemization[31] has been encountered using the Anderson test. t-Butyloxycarbonyl amino acid pentachlorophenyl esters[32] have been found to be useful for rapid peptide synthesis[33] without the isolation of the intermediate peptides. The pentachlorophenyl activated ester has also found great utility for the synthesis of linear polypeptides of a known repeating sequence of amino acids[34-37]. Other coupling agents have been used to form

sequential polypeptides; these include the p-nitrophenyl ester[38-40] and the tetraethylphosphite reagent[41]. A comparative study of the polycondensation of some tripeptides using the various activation methods (tetraethylpyrophosphite, bis(o-phenylene)pyrophosphite, dicyclohexylcarbodiimide and the N-hydroxysuccinimide ester) has appeared[42]. There is a distinct possibility of forming cyclic polypeptides during the polymerization process used for preparing a sequential polypeptide. Thus, studies comparing the various methods used to prepare the cyclic decapeptide antamanide are valuable to polymer chemists. Of the various methods the best yield of cyclic material was obtained using the thiophenyl ester[43]. The fragment condensation method of peptide synthesis has been utilized for the synthesis of Ribonuclease A[44-48]; the fragments were formed through the use of N-carboxyanhydrides, N-thiocarboxyanhydrides, and N-hydroxysuccinimide esters, the latter being routinely employed for incorporation of the amino-terminal acid of all fragments and also for the introduction of asparagine, serine, or threonine. Fragments were coupled by the azide method, thereby overcoming difficulties due to racemization. The fragment condensation method has also been used to synthesize Thyrocalcetonin[49-50].

Solid-phase Peptide Synthesis - This technique of peptide synthesis has received much attention. Its promise as a rapid method for preparing large, biologically active molecules has been fully demonstrated by the synthesis of Ribonuclease A by Merrifield[51]. Other peptides which have been prepared by this method are the B-chains of human and bovine insulin[52], antamanide[53] and the cyclic dodecadepsipeptide, valinomycin[54]. In each case the BOC amino acids were coupled to the resin-bound peptide by dicyclohexylcarbodiimide. Modifications of the Merrifield method are noted in the synthesis of Fibrinopeptide A[55] where BOC amino acid pentachlorophenyl esters[32] were used for extending the peptide chain on the insoluble resin. In the synthesis of some pteroyl-$(\gamma$-L-glutamyl$)_n$-L-glutamic acids[56] the mixed anhydride method was used for the peptide coupling.

A problem in the solid phase method of peptide formation is the synthesis of peptides containing deletions of some of the amino acid residues due to incomplete reaction during the coupling stages. A procedure devised to obviate this difficulty involves acetylation of any uncoupled amine with 3-nitrophthalic anhydride[57]. By this method the desired polypeptide can be separated from the lower 3-nitro-2-carboxybenzoyl peptides. An interesting non-destructive method for the determination of completeness of coupling reactions in solid phase peptide synthesis has been

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References

1. G.T. Hardy, Amino-Acids and Peptides, Ann. Reports, 65, 509 (1968).
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4. J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis W.H. Freeman, San Francisco, 1969.
5. IUPAC-IUB. Comm. Biochem. Nomenclature (USA), Biopolymers, 8 161 (1969).
6. P. Sieber and B. Iselin, Helv. Chim. Acta., 52, 1525 (1969).
7. K.P. Polzhofer, Tetrahedron Letters, 2305 (1969).
8. H. Zahn and K. Hammerstroem, Chem. Ber., 102, 1048 (1969).
9. C.J. Gray, A.M. Khoujah, Tetrahedron Letters, 2647 (1969).
10. S. Sakakisbara, I. Honda, K. Takada, M. Miyoshi and T. Ohnishi, Bull. Chem. Soc. Japan, 42, 809 (1969).
11. S. Sakakibara, I. Honda, M. Naruse and M. Kanaoka, Experientia, 25, 576 (1969).
12. E. Klieger, Ann. Chem., 724, 204 (1969).
13. F. Vandesande, Bull. Soc. Chim. Belg., 78, 395 (1969).
14. T. Wieland and A. Sieber, Ann. Chem., 722, 222 (1969).
15. T. Wieland and A. Sieber, Ann. Chem., 727, 121 (1969).
16. M.A. Tilak and C.S. Hollinden, Tetrahedron Letters, 391 (1969).
17. F. Chillemi and R.B. Merrifield, Biochem., 8, 4344 (1969).
18. K.P. Polzhofer, Tetrahedron, 25, 4127 (1969).
19. B.J. Johnson and P.M. Jacobs, Chem. Commun., 73 (1968).
20. B.J. Johnson and P.M. Jacobs, J. Org. Chem., 33, 4524 (1968).
21. B.J. Johnson and D.E. Tracey, J. Pharm. Sci., 58, 1299 (1969).
22. B.J. Johnson and D.S. Rea, unpublished results.
23. B.J. Johnson, J. Org. Chem., 34, 1178 (1969).

24. B.J. Johnson and T.A. Reuttinger, J. Org. Chem., 35, 255 (1970).
25. R. Camble, R. Garner and G.T. Young, J. Chem. Soc., (C), 1911 (1969).
26. V.A. Shibnev, T.P. Chuvaeva, G.A. Martynova and K.R. Poroshin, Izu. Akad. Nauk. SSSR Ser. Khim., 637 (1969).
27. A. Kapoor, L.W. Gerencser and W.R. Koutnik, J. Pharm. Sci., 58, 281 (1969).
28. K.T. Poroshin, A.B. Zegel'man and T. Yusupov, Dokl. Akad. Nauk. Tadzh. SSR., 12, 20 (1969).
29. K.T. Poroshin, Sh. Kh. Khalikov and V.A. Shibnev. Dokl. Akad. Nauk. Tadzh. SSR, 12, 26 (1969).
30. A. Kapoor and L.W. Gerencsen, J. Pharm. Sci., 58, 976 (1969).
31. J. Kovacs, L. Kosfaludy, M.Q. Ceprini and R.H. Johnson, Tetrahedron, 25, 2555 (1969).
32. B.J. Johnson and E.G. Trask, J. Org. Chem., 33, 4521 (1968).
33. B.J. Johnson, J. Pharm. Sci., 58, 1042 (1969).
34. B.J. Johnson, J. Chem. Soc., (C), 3008 (1968).
35. B.J. Johnson, J. Chem. Soc., (C), 1412 (1969).
36. B.J. Johnson and E.G. Trask, J. Chem. Soc., (C), 2644 (1969).
37. J. Kovacs, G.N. Schmit and B.J. Johnson, Can. J. Chem., 47, 3690 (1969).
38. E. Dellacherie and J. Neen, Bull. Soc. Chim. Fr., 1218 (1969).
39. F.H.C. Stewart, Aust. J. Chem., 22, 1291 (1969).
40. S. Takahashi, Bull. Chem. Soc. Jap., 42, 521 (1969).
41. V.A. Shibnev and A.V. Lazareva, Izu. Akad. Nauk. SSSR, Ser Khim., 398 (1969).
42. E. Heidemann and H.W. Nill, Z. Naturforsch., (B), 24, 843 (1969).
43. T. Wieland, J. Faesel and W. Konz, Ann. Chem., 722, 197 (1969).
44. R.G. Denkewalter, D.F. Veber, F.W. Holly and R. Hirschmann J. Amer. Chem. Soc., 91 502 (1969).
45. R.G. Stachan, W.J. Paleveda Jr., R.F. Nutt, R.A. Vitali, D.F. Veber, M.J. Dirkinson, V. Garsky, J.E. Deak, E. Walton, S.R. Jenkins, F.W. Holly, R. Hirschmann, J. Amer. Chem. Soc., 91, 503 (1969).
46. S.R. Jenkins, R.F. Nutt, R.S. Dewey, D.F. Veber, F.W. Holly, W.J. Paleveda Jr., T. Lanza Jr., R.G. Strachan, E.F. Schoenewaldt, H. Barkemeyer, M.J. Dickinson, J. Sondey, R. Hirschmann, E. Walton, J. Amer. Chem. Soc., 91, 505 (1969).
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56. C.L. Krumdieck and C.M. Baugh. Biochem., 8, 1568 (1969).
57. T. Wieland, C. Birr and H. Wissenbach, Angew. Chem., Int. Ed., 8, 764 (1969).
58. L.C. Dorman, Tetrahedron Letters, 2319 (1969).
59. W. Pereira, V.A. Close, E. Jellum, W. Patton, B. Halpern, Aust. J. Chem., 22, 1377 (1969).
60. T. Mizoguchi, K. Shigezane and N. Takamuro, Chem. Pharm. Bull. (Tokyo), 17, 411 (1969).
61. B. Green, L.R. Garson, J. Chem. Soc., (C), 401 (1969).

Chapter 28. Recent Methods in Peptide Synthesis

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Introduction - This review is based primarily on papers published in 1969, and it is selective for the sake of brevity. It is limited to purely organic synthetic methods; structure-activity relationships are discussed in other chapters.

Books and Review Articles - A number of book and review articles have appeared during the last year. Advances in the chemical synthesis of peptides were reviewed by Hardy[1], Katsoyannis[2] and Young[3]. The description of the experimental details for the Merrifield method of peptide synthesis has been published by Stewart[4]. It is also noted that the tentative rules[5] for the abbreviated nomenclature of synthetic peptides have also been published.

Protecting Groups - A number of new protecting groups, some of which are variations of those already in use, have been introduced. Further work has appeared on the very interesting aralkyloxycarbonylamino protecting groups, with the synthesis of 2-(p-biphenylyl)isopropoxycarbonyl amino acids and their activated esters[6]. The N-protecting group was introduced by the use of 2-(p-biphenylyl)isopropylphenyl carbonate, a reagent which is stable at 0°. The advantage of this group is its ready removal by dilute acetic acid even in the presence of the t-butyloxycarbonyl (BOC) group. The useful derivatives, BOC-γ-benzyl-glutamic acid and BOC-β-benzyl-aspartic acid, have been prepared by an improved method[7]; also various S-substituted derivatives of cysteine have been converted to their corresponding BOC compounds[8] using the Schnabel method. An ion exchange method for selectively removing the BOC group, even in the presence of the tert-butyl ester, has been reported[9]. Other N-protecting groups which have received attention this year are the tert-amyloxycarbonyl[10], prepared from the corresponding chloroformate, and p-methoxybenzyloxycarbonyl, prepared either from its chloroformate or the 2, 4, 5-trichlorophenylcarbonate[11-12]. Both groups can be deblocked under mild conditions. The use of the p-methoxybenzyloxy-carbonyl protecting group has been illustrated in a new synthesis of oxytocin[13]. A new S-protecting group for cysteine, the β,β-diethoxycarbonylethyl residue has been suggested[14] and its use has been illustrated in a new synthesis of glutathione[15]. An improved synthesis of N(im)-benzylhistidine has been reported[16], without the use of sodium in liquid ammonia. The newer method of protecting the

imidazole ring of histidine with the dinitrophenyl group has been utilized in the synthesis of sequences of human hemoglobin β chain[17] using the solid phase technique for peptide synthesis. Also, the 2,2,2-trifluoro-1-(benzyloxycarbonylamino)ethyl group[18] has been used as a method of protecting the hydroxy functions of serine and threonine.

A new amino acid carboxyl protecting group, the 4-(methylthio)phenyl ester, has been developed[19]. These esters are easily prepared by the N,N'-dicyclohexylcarbodiimide method, and the BOC and carbobenzoxy protecting groups can be easily removed in their presence. The attractive feature of this protective ester is its facile conversion, by oxidation and without racemization[20], to the activated 4-(methylsulfonyl)phenyl ester. This method has been used successfully for peptide[21], polypeptide[22] and O-depsipeptide synthesis[23]. The preparation and properties of a number of 4-(methylthio)phenyl esters of amino acids have been reported[24], including new oxidation conditions which permit the method of protection and then activation to be applicable to nearly all of the usual protecting groups and the commonly occurring amino acids, with the exception of those containing sulfur. The preparation and properties of some 4-picolyl esters of amino acids have been reported [25]. These esters are cleaved by cold alkali, by catalytic hydrogenation, by sodium in liquid ammonia and by electrolytic reduction. A new procedure for the facilitation of peptide synthesis is also reported in which the carboxyl-terminal residue is incorporated as its 4-picolyl ester; after each coupling reaction the product is separated by extraction into an acidic phase.

Peptide Bond Formation - Most methods described for peptide formation have been dependent upon enhancement of the reactivity of one of the functional groups of the amino acid. Usually the carboxyl group of the amino acid is activated and, where possible, the activation of peptide terminal carboxyl groups is avoided because of dangers of racemization. Halogenated phenyl esters, especially the trichloro[26] and pentachlorophenyl esters[27-30], have received continuing attention. With the latter activated ester, no racemization[31] has been encountered using the Anderson test. t-Butyloxycarbonyl amino acid pentachlorophenyl esters[32] have been found to be useful for rapid peptide synthesis[33] without the isolation of the intermediate peptides. The pentachlorophenyl activated ester has also found great utility for the synthesis of linear polypeptides of a known repeating sequence of amino acids[34-37]. Other coupling agents have been used to form

sequential polypeptides; these include the p-nitrophenyl ester[38-40] and the tetraethylphosphite reagent[41]. A comparative study of the polycondensation of some tripeptides using the various activation methods (tetraethylpyrophosphite, bis(o-phenylene)pyrophosphite, dicyclohexylcarbodiimide and the N-hydroxysuccinimide ester) has appeared[42]. There is a distinct possibility of forming cyclic polypeptides during the polymerization process used for preparing a sequential polypeptide. Thus, studies comparing the various methods used to prepare the cyclic decapeptide antamanide are valuable to polymer chemists. Of the various methods the best yield of cyclic material was obtained using the thiophenyl ester[43]. The fragment condensation method of peptide synthesis has been utilized for the synthesis of Ribonuclease A[44-48]; the fragments were formed through the use of N-carboxyanhydrides, N-thiocarboxyanhydrides, and N-hydroxysuccinimide esters, the latter being routinely employed for incorporation of the amino-terminal acid of all fragments and also for the introduction of asparagine, serine, or threonine. Fragments were coupled by the azide method, thereby overcoming difficulties due to racemization. The fragment condensation method has also been used to synthesize Thyrocalcetonin[49-50].

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38. E. Dellacherie and J. Neen, Bull. Soc. Chim. Fr., 1218 (1969).
39. F.H.C. Stewart, Aust. J. Chem., 22, 1291 (1969).
40. S. Takahashi, Bull. Chem. Soc. Jap., 42, 521 (1969).
41. V.A. Shibnev and A.V. Lazareva, Izu. Akad. Nauk. SSSR, Ser Khim., 398 (1969).
42. E. Heidemann and H.W. Nill, Z. Naturforsch., (B), 24, 843 (1969).
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46. S.R. Jenkins, R.F. Nutt, R.S. Dewey, D.F. Veber, F.W. Holly, W.J. Paleveda Jr., T. Lanza Jr., R.G. Strachan, E.F. Schoenewaldt, H. Barkemeyer, M.J. Dickinson, J. Sondey, R. Hirschmann, E. Walton, J. Amer. Chem. Soc., 91, 505 (1969).
47. D.F. Veber, S.L. Varga, J.D. Milkowski, H. Joshua, J.B. Conn, R. Hirschmann, R.G. Denkewalter, J. Amer. Chem. Soc. 91, 506 (1969).

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50. B. Kamber, W. Rittel, Helv, Chim. Acta, 52, 1074 (1969).
51. B. Gutte and R.B. Merrifield, J. Amer. Chem. Soc., 91, 501 (1969).
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54. B.F. Gisin, R.B. Merrifield and D.C. Tostevon, J. Amer. Chem. Soc., 91, 2691 (1969).
55. B.J. Johnson and W.P. May, J. Pharm. Sci., 58, 1568 (1969).
56. C.L. Krumdieck and C.M. Baugh. Biochem., 8, 1568 (1969).
57. T. Wieland, C. Birr and H. Wissenbach, Angew. Chem., Int. Ed., 8, 764 (1969).
58. L.C. Dorman, Tetrahedron Letters, 2319 (1969).
59. W. Pereira, V.A. Close, E. Jellum, W. Patton, B. Halpern, Aust. J. Chem., 22, 1377 (1969).
60. T. Mizoguchi, K. Shigezane and N. Takamuro, Chem. Pharm. Bull. (Tokyo), 17, 411 (1969).
61. B. Green, L.R. Garson, J. Chem. Soc., (C), 401 (1969).

Chapter 30. Alkaloids

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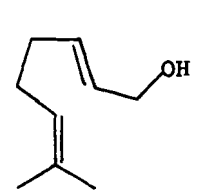
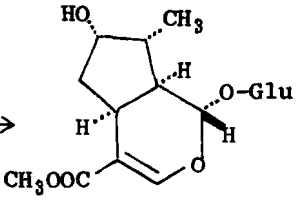
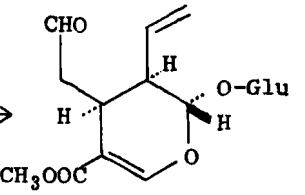
Indole Alkaloids - Full papers have appeared on the role of loganin, secologanin, and vincoside as precursors for the indole alkaloids, and the following sequence is now established in Vinca rosea:[1]



Geraniol

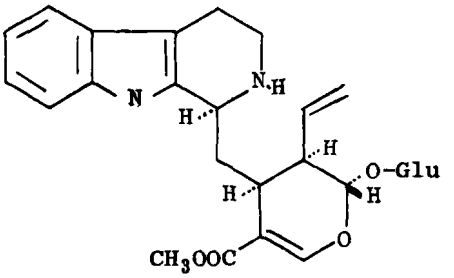


Loganin



Secologanin

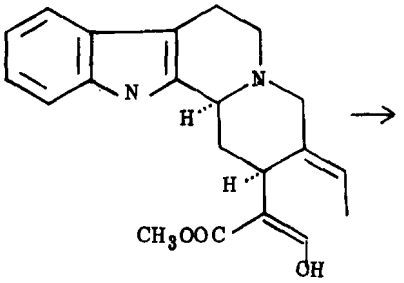
Tryptamine



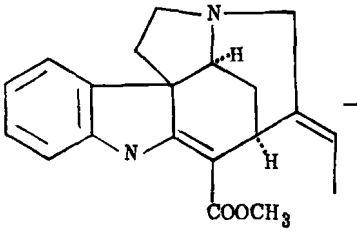
Vincoside

Corynanthe type alkaloids.

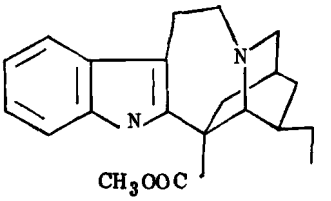
Furthermore, substantial evidence has now been presented for the transformation sequence in V. rosea: Corynanthe → Strychnos → Aspidosperma → Iboga. Thus feeding labeled geissoschizine resulted in formation of labeled akuammicine and coronaridine:[2,3]



Geissoschizine



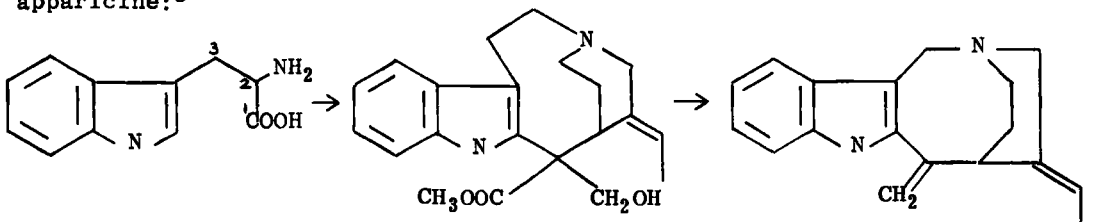
Akuammicine



Coronaridine

When glycine-2-C^{14} was fed to Cephaelis acuminata, the label was very efficiently incorporated into the C_{9-10} unit of cephaeline (an isoquinoline alkaloid); but when sodium acetate-2-C^{14} was fed, the cephaeline obtained was of very low activity. The oxidation state of a two carbon compound may, therefore, exert a major effect on its utilization in biosynthesis.[4]

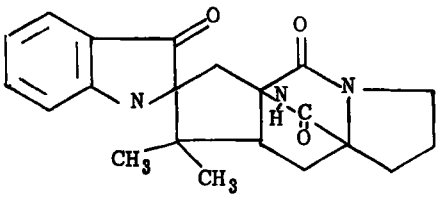
Work with Aspidosperma pyricollum has demonstrated that the biosynthesis of apparicine from tryptophane occurs by loss of C-2 and retention of C-3. Additionally, labeled stemmadenine was readily incorporated into apparicine:[5]



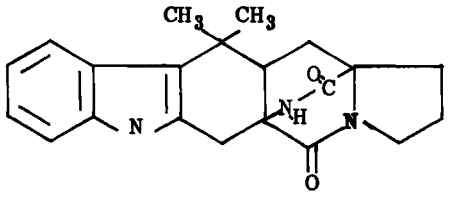
Stemmadenine Apparicine

The incorporation of labeled sweroside into reserpinine and quinine has also been achieved.[6]

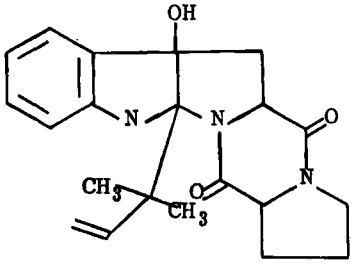
Newly isolated indoles of interest are the fungal alkaloids brevianamide-A, deoxybrevianamide-A and brevianamide-E from Penicillum brevicompactum;[7] the terpenoidal alkaloids cyclomahanimbine and mahanimbine from Murraya koenigii (Rutaceae);[8] and the Gardneria alkaloid gardnutine.[9]



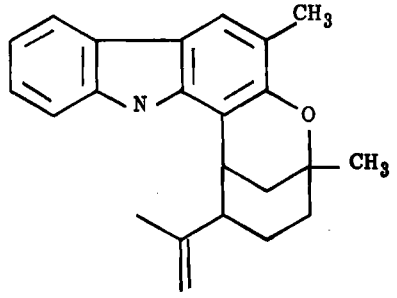
Brevianamide-A



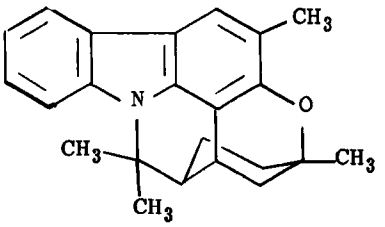
Deoxybrevianamide-A



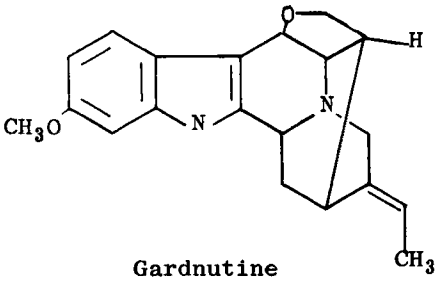
Brevianamide-E



Cyclomahanimbine



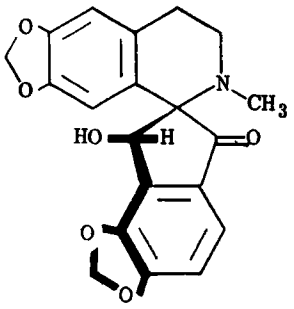
Mahanimbine



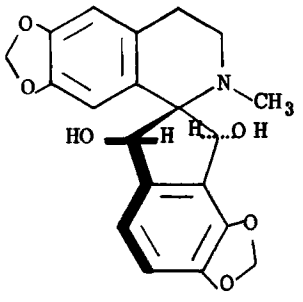
Gardnutine

New syntheses of indole alkaloids that have come out in communication form are those for homoeburnamenine,[10] geissoschizoline,[11] tubotaiwine[11], ibogamine,[12] epiibogamine,[13] isoajmaline,[14] dihydrocorynantheol,[15] and vincamine.[16] Dihydrohunterburnine α-methochloride, 10-methoxydihydrocorynantheol and ochrosandwine have been synthesized from quinine, and the absolute configuration of these indole bases has been established.[17]

Isoquinoline and Related Alkaloids - Two new spirobenzylisoquinoline alkaloids are sibiricine and ochrobirine.[18]

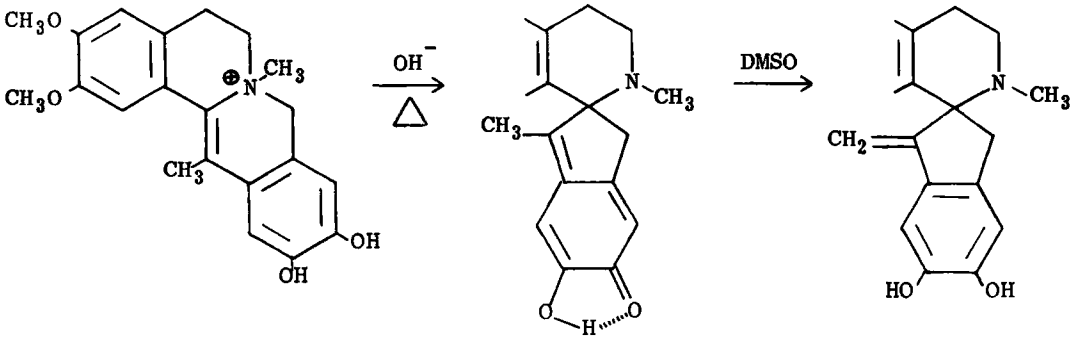


Sibiricine

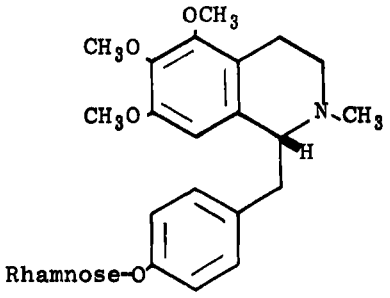


Ochrobirine

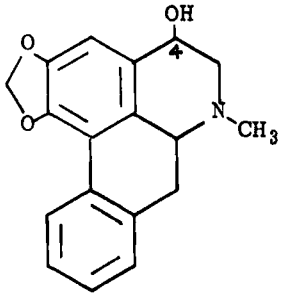
A synthesis of the spirobenzylisoquinoline system present in ochotensimine has been carried out starting from a quaternary diphenolic protoberberine salt, and this rearrangement may duplicate in part the biogenetic process:[19]



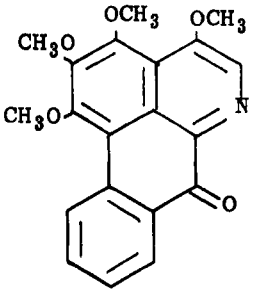
A new glycosidic benzylisoquinoline base is veronamine, where the carbohydrate moiety is rhamnose.[20] The oxoaporphine alkaloid liriodenine has shown significant cytotoxic activity,[21] and the first quaternary oxoaporphine alkaloid has been found in Papaver orientale.[22] An aporphine and an oxoaporphine oxygenated at C-4 are steporphine and imenine respectively.[23]



Veronamine



Steporphine



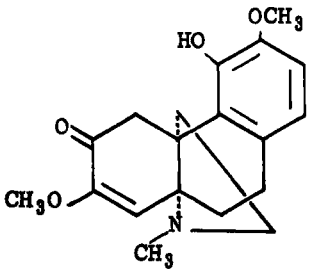
Imenine

The positions of the substituents in ring D of such protoberberine alkaloids as mecambridine and orientalidine have been shown to be 10,11,12 rather than 9,10,11.[24] A review summarizing the UV spectra of protoberberines has come out.[25]

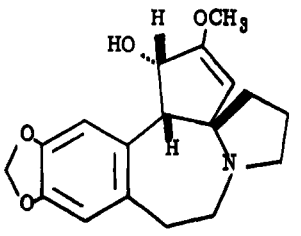
A new book which should prove a useful reference manual entitled: "The Chemistry of the Isoquinoline Alkaloids", authored by Prof. T. Kametani, has appeared. It is published by the Hirokawa Publishing Co., Tokyo, and is a complete listing with physical constants of all isoquinoline alkaloids. Publication was in 1968.

The total synthesis of the alkaloid cepharamine has been achieved.[26] Finally, a complete biogenetic sequence from tyrosine to mescaline can now be defined.[27,28]

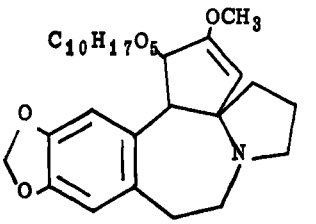
Cephalotaxus Alkaloids - Four alkaloids of novel structures have been isolated from Cephalotaxus harringtonia var. drupacea.[29] These are cephalotaxine, harringtonine, and the unnamed bases I and II. Harringtonine, which is an ester of cephalotaxine, has shown significant inhibitory activity against the experimental lymphoid leukemia L-1210 and P-388 in mice.



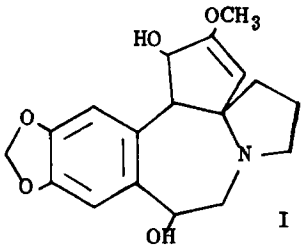
Cepharamine



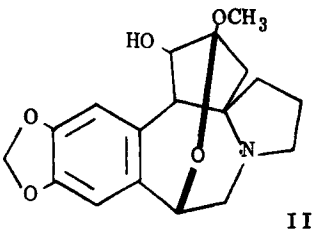
Cephalotaxine



Harringtonine



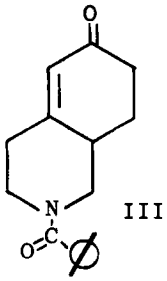
I



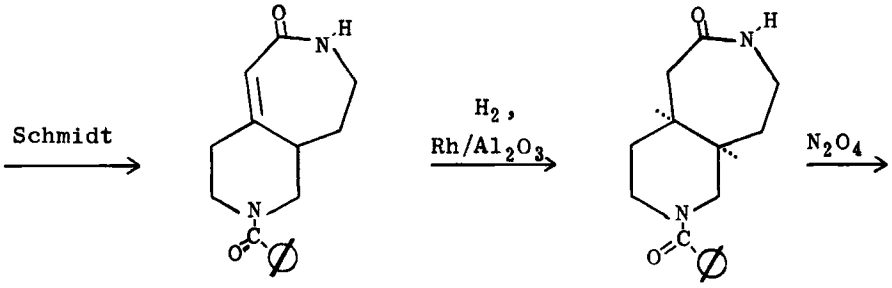
II

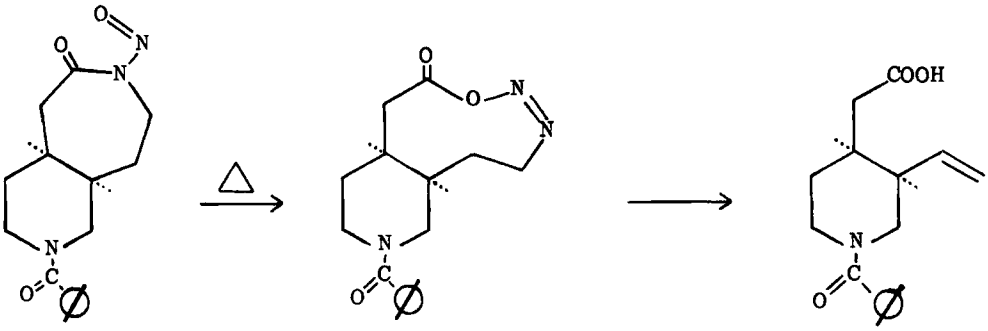
Upon base hydrolysis, harringtonine yields cephalotaxine and an unidentified acid. A single crystal x-ray study of cephalotaxine methiodide has revealed the stereochemistry and absolute configuration.[30]

Quinoline Alkaloids - An elegant stereoselective total synthesis of quinine and quinidine has been reported.[31,32] The unsaturated ketoamide III was converted to N-benzoylmeroquinene as shown. Condensation of the methyl ester of this acid with 6-methoxylepidyllithium followed by the transformations indicated furnished quinine and the diastereoisomeric quinidine.

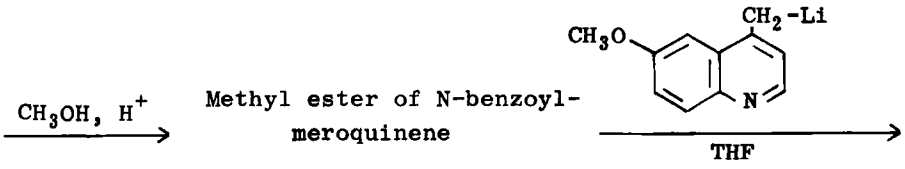


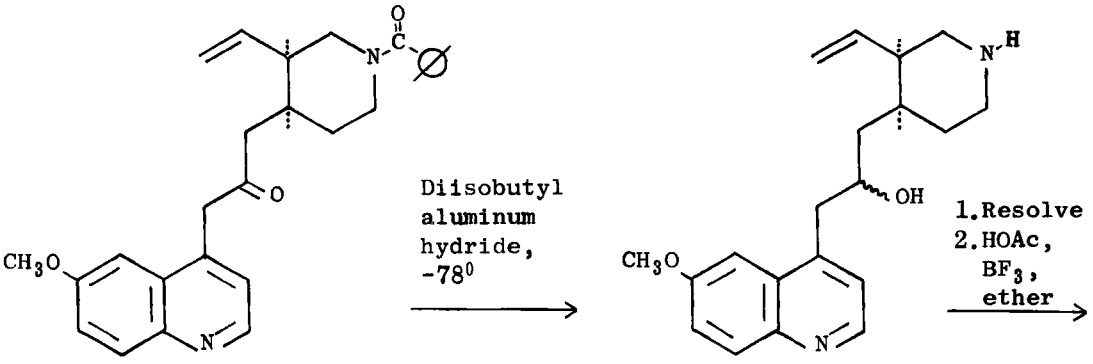
III

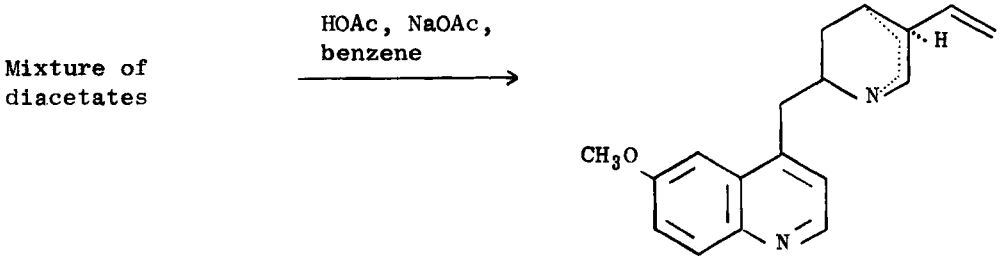


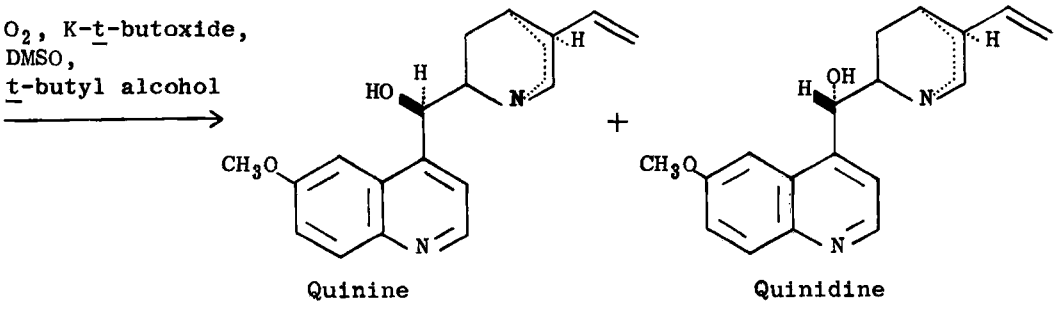


N-Benzoyl-meroquinene



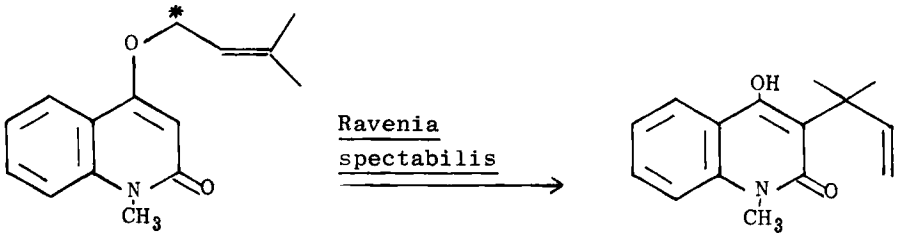

Diisobutyl aluminum hydride, -78°
1. Resolve
2. HOAc, BF3, ether


Mixture of diacetates
HOAc, NaOAc, benzene

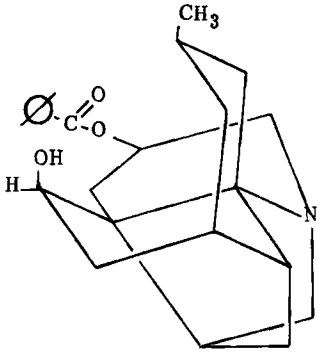

O2, K-t-butoxide, DMSO, t-butyl alcohol
Quinine
Quinidine

A partial synthesis of quinine and quinidine has also been reported.[33]

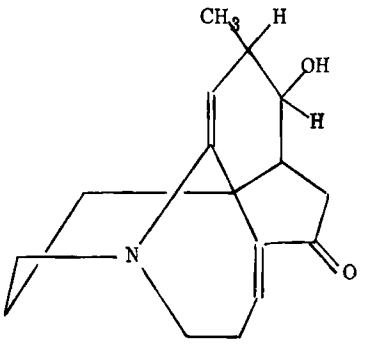
A Claisen rearrangement has been shown to occur during the biogenesis of the bicyclic alkaloid ravenoline:[34]



Lycopodium Alkaloids - A full account of the Wiesner synthesis of annotinine has now appeared.[35] New alkaloids from Lycopodium species are alopecurine[36] and alolycopine.[37] The stereochemistry of the C-3 to C-4 bond in serratinine is beta, as shown by x-ray analysis, so that the structure of this interesting alkaloid is now completely elucidated.[38]

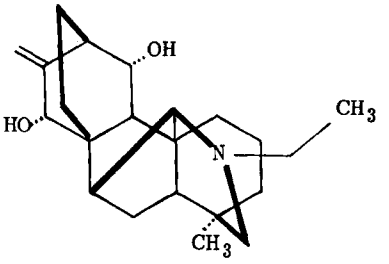


Alopecurine



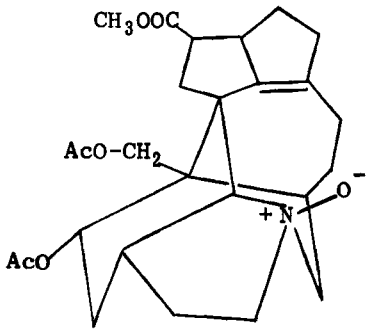
Alolycopine

Diterpene Alkaloids - A rigorous, purely chemical, structure proof for aconitine and delphinine has appeared.[39,40] The delphinium alkaloid denudatine has been shown by x-ray analysis to be as indicated. It is thus the first authenticated representative of the skeletal type previously postulated as possible intermediates in the biogenetic transformation of the atisine into the aconitine skeleton.[41,42]

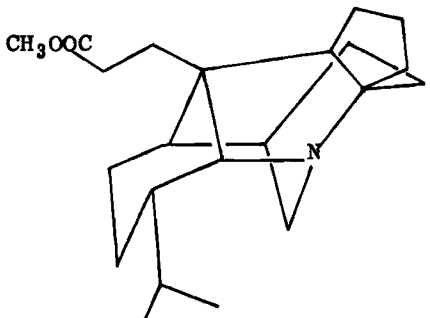


Denudatine

Daphniphyllum Alkaloids - A revised structure for macrodaphnine has been presented, based on x-ray data.[43] Methyl homodaphniphyllate has been isolated from nature. The latter compound may be a biogenetic intermediate between the more complex daphniphylline and yuzurimine.[44]

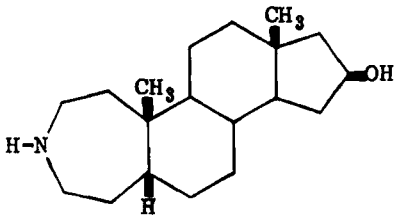


Macrodaphnine



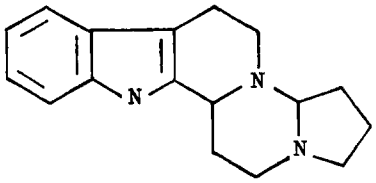
Methyl homodaphniphyllate

Steroidal Alkaloids - Samanine, a new alkaloid from the skin gland secretions of the salamander has been characterized and synthesized.[45] The structures for solanocapsine and dihydrojervine[46,47] have been revised, and the syntheses of pachysandrine-A, B and C have been carried out.[48]



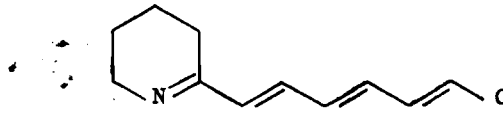
Samanine

Elaeocarpus Alkaloids - New compounds from this source continue to be isolated,[49] and the new alkaloid elaeocarpidine has already been synthesized.[50]



Elaeocarpidine

Pyridine Alkaloids - From Streptomyces strain FFD-101, the new unstable base nigrifactin has been isolated. It affects the blood pressure and possesses antihistamine activity.[51]



Nigrifactin

REFERENCES

1. A.R. Battersby, A.R. Burnett and P.G. Parsons, J. Chem. Soc. (C), 1187 and 1193 (1969).
2. A.I. Scott, P.C. Cherry and A.A. Qureshi, J. Am. Chem. Soc., 91, 4932 (1969).
3. A.R. Battersby and E.S. Hall, Chem. Commun., 793 (1969).
4. A.K. Garg and J.R. Gear, Tetrahedron Letters, 4377 (1969).
5. J.P. Kutney, V.R. Nelson and D.C. Wigfield, J. Am. Chem. Soc., 91, 4278 and 4280 (1969).
6. H. Inouye, S. Ueda and Y. Takeda, Tetrahedron Letters, 407 (1969).
7. A.J. Birch and J.J. Wright, Chem. Commun., 644 (1969).
8. S.P. Kureel, R.S. Kapil and S.P. Popli, Tetrahedron Letters, 3857 (1969); and Chem. Commun., 1120 (1969).
9. S. Sakai, A. Kubo and J. Haginiwa, Tetrahedron Letters, 1485 (1969).
10. K.J. Gilson and J.A. Saxton, Chem. Commun., 799 (1969).
11. B.A. Dadson and J. Harley-Mason, Chem. Commun., 665 (1969).
12. M. Ikezaki, T. Wakamatsu and Y. Ban, Chem. Commun., 88 (1969).
13. P. Rosenmund, W.H. Haase and J. Bauer, Tetrahedron Letters, 4121 (1969).
14. M. Mashino and Y. Sato, Tetrahedron Letters, 901, 905 (1969).
15. F.E. Ziegler and J.G. Sweeny, Tetrahedron Letters, 1097 (1969).
16. K.H. Gibson and J.E. Saxton, Chem. Commun, 1490 (1969).
17. Y.K. Sawa and H. Matsamura, Tetrahedron, 25, 5319 and 5329 (1969).
18. R.H.F. Manske, R. Rodrigo, D.B. MacLean, D.E.F. Gracey and J.K. Saunders, Can. J. Chem., 47, 3586 and 3589 (1969).
19. M. Shamma and C.D. Jones, J. Am. Chem. Soc., 91, 4009 (1969).
20. M. Shamma, M.G. Kelly and Sr. M.A. Podczasy, Tetrahedron Letters, 4951 (1969).
21. D. Warthen, E.L. Gooden and M. Jacobson, J. Pharm. Sc., 58, 637 (1969).
22. V. Preininger, J. Hrbek jun., Z. Samek and F. Šantavý, Archiv. Pharm., 302, 808 (1969).
23. J. Kunitomo, Y. Okamoto, E. Yuge and Y. Nagai, Tetrahedron Letters, 3287 (1969); and M.D. Glick, R.E. Cook, M.P. Cava, M. Srinivasan, J. Kunitomo and A.I. daRocha, Chem. Commun., 1217 (1969).
24. V. Preininger, V. Simanek and F. Šantavý, Tetrahedron Letters, 2109 (1969).
25. M. Shamma, M.J. Hillman and C.D. Jones, Chem. Rev., 69, 779 (1969).
26. Y. Inubushi, T. Ibuka and M. Kitano, Tetrahedron Letters, 1611 (1969).
27. J. Lundstrom and S. Agurell, Tetrahedron Letters, 3371 (1969).
28. A.G. Paul, K.L. Khanna, H. Rosenberg and M. Takido, Chem. Commun., 838 (1969).
29. R.G. Powell, D. Weisleder, C.R. Smith, Jr., and I.A. Wolff, Tetrahedron Letters, 4081 (1969).
30. D.J. Abraham, R.D. Rosenstein and E.L. McGandy, Tetrahedron Letters, 4085 (1969).
31. M. Uskoković, J. Gutzwiller and T. Henderson, J. Am. Chem. Soc., 92, 204 (1970).
32. G. Gutzwiller and M. Uskoković, J. Am. Chem. Soc., 92, 204 (1970).
33. M. Gates, B. Sugavanam and W.L. Schreiber, J. Am. Chem. Soc., 92, 205 (1970).

34. T.R. Chamberlain, J.F. Collins and M.F. Grundon, Chem. Commun., 1269 (1969).
35. K. Wiesner, L. Poon, I. Jirkovsky and M. Fishman, Can. J. Chem., 47, 433 (1969).
36. W.A. Ayer, B. Altenkirk, N. Masaki and S. Valverde-Lopez, Can. J. Chem., 47, 2449 (1969).
37. W.A. Ayer and B. Altenkirk, Can. J. Chem., 47, 2457 (1969).
38. N. Nishio, T. Fujiwara, K. Tomita, H. Ishii, Y. Inubushi and T. Harayama, Tetrahedron Letters, 861 (1969).
39. K. Wiesner, W.-L. Kao and E.W.K. Jay, Can. J. Chem., 47, 2734 (1969).
40. K. Wiesner, W.-L. Kao and J. Santroch, Can. J. Chem., 47, 2431 (1969).
41. M. Götz and K. Wiesner, Tetrahedron Letters, 4369 (1969).
42. M. Götz, K. Wiesner and F. Brisse, Tetrahedron Letters, 4373 (1969).
43. T. Nakano and B. Nilsson, Tetrahedron Letters, 2883 (1969).
44. M. Toda, S. Yamamura and Y. Hirata, Tetrahedron Letters, 2585 (1969).
45. G. Habermehl and A. Haaf, Ann. Chem., 722, 155 (1969).
46. H. Ripperger and K. Schreiber, Ann. Chem., 723, 159 (1969).
47. T. Masamune, A. Murai, H. Ono, K. Orito and H. Sugimo, Tetrahedron Letters, 255 (1969).
48. T. Kikuchi, T. Nishinaga and Y. Yoshimura, Tetrahedron Letters, 1679 (1969).
49. S.R. Johns, J.A. Lamberton, A.A. Sioumis and R.I. Willing, Aust. J. Chem., 22, 775 (1969).
50. J. Harley-Mason and C.G. Taylor, Chem. Commun., 281 (1969).
51. T. Terashima, Y. Kuroda and Y. Kaneko, Tetrahedron Letters, 2535 (1969).

Chapter 31. Nucleosides and Nucleotides

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Two years have passed since this series reviewed a year's (1967) progress in the chemistry of nucleosides and nucleotides. During this time there has been no decline in interest and activity in this field; the enormous number of organic chemical publications parallels the intense activity of biochemists and biologists in nucleic acid research. This review surveys chemical work published during 1968 and 1969, with particular emphasis on synthetic aspects. The first volume of a very useful series of selected synthetic procedures in nucleic acid chemistry was published during this period.[1]

Direct Synthesis of Nucleosides - A number of new thio[2,3]-, amino[4]-, and branched[5-11]- sugar nucleosides of adenine (including 3'-thioadenosine[3], 2'-, and 3'-C-methyladenosines[5,6]) were synthesized via condensation of a chloromercuri-6-acylaminopurine with the appropriate new blocked sugar halides or 1-acetates (the latter in the presence of $TiCl_4$). In many cases both anomers were obtained in these condensation reactions, although a "participating" acyloxy group at C-2 of the sugar reactant usually favored the trans nucleosidic product. Condensations of the chloromercuripurine with halogenoses having "nonparticipating" isopropylidene blocking groups[12-14] appeared to proceed predominantly with retention of configuration, leading to the conclusion[12,13] that these reactions (involving heavy metal salts) are S_N1 type, and that steric hindrance from the 2,3-O-isopropylidine group favors trans configuration for both the halogenose and the nucleosidic product. Anomeric mixtures of both the (blocked) N-7 and N-9 nucleosides were obtained when the silver salt of hypoxanthine was reacted with pure α-acetobromoglucose[15,16], while from the reactions of anomeric 2',3',5'-tri-O-acyl-D-ribofuranosyl halides with the chloromercuri derivatives of 3-benzylhypoxanthine and 9-propenylhypoxanthine only the β-anomers of the corresponding (blocked) N-7- and N-1-ribofuranosyl derivatives, respectively, were isolated[17], thus showing closer adherence to the "trans rule" as well as dependence of the site of alkylation on the position of the first ring-N-substituent. In contrast, when the free base 9-propenylhypoxanthine reacted with two differently blocked anomeric ribofuranosyl halides in dimethylacetamide, the α/β anomer ratios of the N-7 ribofuranoside derivatives obtained appeared to be inversely related to those of the halogenose reactants[17], indicating that the condensation in absence of metal salts may have proceeded via an S_N2-type displacement by the most basic ring-nitrogen. At least partial operation of this type of mechanism may be indicated in the condensations of benzyl- and benzoyl-blocked ribofuranosyl halides with 5,6-dimethylbenzimidazole in dioxane, leading to the blocked α and β "ribazoles"[18,19]. However, condensation of 7-pivaloyloxymethyl-adenine with anomeric 2,5-di-O-benzoyl-3-deoxy-D-ribofuranosyl bromide in acetonitrile led, after deblocking, only to the 3-β- nucleoside, "3-isocordycepin"[20].

Acid-catalyzed fusion of tetra-O-acetyl-D-ribofuranose with purine yielded, after deblocking, the 9-β- and 7-β-nucleosides nebularine and isonebularine.[21] The corresponding 9-β- and 7-β-xylofuranosides and ribopyranosides were obtained in a similar manner[21], while only the 9-β-isomer was obtained in the preparation of glucopyranosylpurine[21] and of several 6-methylpurine nucleosides[22] by the fusion procedure. Acid-catalyzed fusion of a blocked 2-deoxysugar 1-acetate with 2-fluoro-6-benzyloxypurine yielded the anomeric N-9-nucleosides.[23] Two additional novel procedures were reported for the synthesis of purine nucleosides, involving acid catalysis. Adenine was converted to the organic solvent-soluble N^6-octanoyl derivative which reacted in an aprotic solvent with blocked ribofuranosyl or ribopyranosyl 1-acetate in the presence of $SnCl_4$ to give, after deacylation, high yields of anomerically pure adenosine and 9-β-ribopyranosyladenine, respectively.[24] The corresponding nucleosides of guanine were prepared from N^2-palmitoylguanine in a similar manner. Formation of the other anomer was not observed.[24] The second procedure involves acid-catalyzed transfer of an acylated glycosyl (or phosphoglycosyl) group, at high temperature in solution, from a pyrimidine nucleoside or nucleotide which has been fully acylated both at the pyrimidine and sugar moiety, to an appropriately protected heterocyclic base (6-benzamidopurine or benzimidazole).[25] Although anomerization occurs and the yields are variable (10-44%), this method may be of practical value due to its simplicity.

Several new purine nucleoside analogs were prepared by direct or total syntheses, including (as representatives of different types) the indole nucleoside "1,3,7-triazaadenosine"[26] carbocyclic (cyclopentyl) analogs of 2'- and 3'-deoxyadenosines,[27] a "reversed nucleoside" in which methyl 2-deoxyriboside is linked through its 5-methylene group to N-9 of adenine[28], and a "homonucleoside" containing a methylene group between the "glycosidic" carbon and N-9 of adenine.[29]

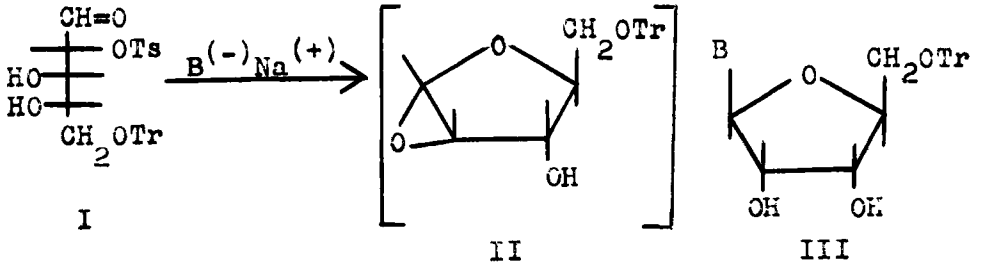
In synthesis of pyrimidine nucleosides, the mercuripyrimidine method was employed for the preparation of 6-methyl and 5,6-dimethyl uridines and cytidines[30], 2-thiouridine and 2-thiocytidine (from the S-acetyl-pyrimidines)[31], as well as the 1-β-D-ribosides of 4-pyrimidinone [31], 2-pyrimidinone[32], and 4,6-pyrimidinedione.[33] In the latter case, O-glycosides were first obtained and these were converted to the nucleosides by $HgCl_2$-catalyzed rearrangement. The influence of the solvent on the course of the coupling reactions (O-vs. N-glycoside formation, anomer ratio) was studied with mercuripyrimidines[34], as was the effect of temperature on the ratio of N_1-, N_3-, O^2, O^4-di-, N_1,O^4-di-, and N_1,N_3-di-glycosides obtained in coupling reactions of the silver salt of uracil.[35] Helferich's mercuric cyanide-nitromethane modification was shown to represent a significant improvement of the mercuri method as it does not require isolation of the mercuripyrimidines; permits the coupling reaction to proceed in a homogenous reaction mixture; and provides high yields of the blocked nucleosides.[36] This procedure gives good results even when the original mercuri procedure presents difficulties or fails altogether. Thus, the blocked nucleosides of uracil, 5-cyano-, 5-nitro-, and 5-fluorouracil were obtained in 70-88% yields.[36] Cytosine and 2-thiocytosine were also synthesized directly without protection of the

amine or sulfur.[37]

An important study [38] on the mechanism of the Hilbert-Johnson reaction demonstrated for the first time the formation of an intermediate glycosylpyrimidinium salt. The reaction of 2-methylthio-4-amino-pyrimidine with tri-O-benzoyl-D-ribofuranosyl chloride in acetonitrile could be stopped at the quaternization step, and the 2-methylthio group of the intermediate could be replaced by an NH_2, OH, or SH group to give, after deprotection, 2,4-diaminopyrimidine riboside, cytosine, or 2-thiocytosine, respectively.[38] Increasingly, the silyl modification of the Hilbert-Johnson reaction appears to be the method of choice for the direct synthesis of a variety of pyrimidine nucleosides[39-44] and their "aza"[45]- and "deaza"[46]-analogs, as well as for the introduction of glycosyl groups into some other heterocyclic bases.[47,48] The coupling reactions of trimethylsilyl pyrimidines with glycosyl halides usually proceed in good to excellent yields under a variety of conditions[39], yielding only the N_1-nucleosides (except in the case of the silyl derivative of 6-methyluracil which gave the N_3-nucleoside[40]-also observed to be formed by the mercuri method[30]), and the products are readily desilylated and isolated. Moreover, if the reaction is conducted in the presence of a metal salt catalyst[39] or in acetonitrile solution [41], the coupling of silylpyrimidines with blocked glycosyl halides having a 2-acyloxy group is reported to result in exclusive or predominant formation of the "trans-nucleoside" anomer.[39] However, when the silylated 5-acetylmercaptouracil reacted with benzoyl-, or p-chlorobenzoyl-blocked ribofuranosyl halides under fusion conditions, the amount of α-nucleoside formed appeared to be related to the amount of the β-anomer present in the halogenose reagent, indicating that the coupling proceeded via a simple S_N2-type displacement involving a single Walden inversion.[49] While the coupling of silylpyrimidines with 2'-deoxyglycosyl halides is usually reported to lead to anomeric mixtures of the nucleosidic products[39,41], a recent study indicated that it is possible to conduct this reaction in a stereoselective manner, to yield either the α or the β 2'-deoxynucleoside, by proper choice of the reaction conditions.[44]

An interesting new procedure was employed in the synthesis of L-adenosine, L-guanosine, L-cytidine, L-uridine, L-5-methyluridine, and L-6-azauridine, involving the reaction of 2-O-tosyl-5-O-trityl-L-arabinose (I) with a suspension of the sodium salt of the appropriate base in DMF, to give 32-92% yields of the 5'trityl derivatives of the corresponding L-β-ribofuranosides (III).[50] The reaction presumably proceeds through an epoxide intermediate (II) resulting from the action of the base on I.[50]

A synthesis of pseudouridine (α- and β- isomers) and of 5-β-D-ribofuranosyluridine has been reported.[51] "Homouridine" and "homocytosine", having a methylene group inserted between N_1 of the pyrimidine and C_1' of the glycosyl group, were synthesized via the ureido derivative of 1-deoxy-1-amino-2,5-anhydro-D-allitol.[52]



I II III

Transformations and Other Reactions of Nucleosides - Several 7-β-D-ribofuranosylpurine derivatives were synthesized by ring closure of appropriately substituted imidazole nucleosides[53,54]. 2-Azaadenosine[55] and 6-azapseudoridine[56] were synthesized via ring opening and ring closure reactions from adenosine and pseudouridine, respectively. Bromination of toyocamycin followed by ring closure gave a tricyclic pyrazolopyrrolopyrimidine nucleoside[57].

A new series of N^6-substituted adenosine (and 7-deazaadenosine) derivatives, related to the "minor nucleoside" N^6-isopentenyladenosine (IPA) present in tRNA and having cytokinin activity, were synthesized via quaternization at N_1 of adenosine with the appropriate R-X and subsequent rearrangement of the R group to the N^6-position[58]. The $N_1 \rightarrow N^6$ rearrangement and various other reactions of the isopentenyl group of IPA were studied[59,60], and a new isomer of IPA (3iPA) was synthesized[61]. Two papers deal with the rearrangement of N^6-(α-aminoacyl) adenines into N-(6-purinyl)amino acids[62,63].

Direct N-amination of various purine nucleosides at N_1 and/or N_7 of the purine moiety was accomplished by treatment with hydroxylamine-O-sulfonic acid with aqueous alkali; the resulting N-aminopurine nucleosides can be readily deaminated by treatment with HNO_2[64]. Selective N-alkylation of uridine and thymidine, to give high yields of the corresponding N_3-ethyl (or methyl) derivatives, was achieved in aqueous solution by treatment with triethyl-(or trimethyl) oxonium fluoroborate; cytidine, adenosine, and guanosine gave no reaction with the reagent[65]. Various new 6-mercapto[66]-, 2,6-hydroxylamino[67]-, and 8-substituted (OH, SH, N_3, NH_2)[68] purine nucleosides were synthesized by standard reactions, starting from the corresponding halopurine nucleosides. Tri-O-acetyl-8-bromoguanosine, on treatment with $POCl_3$, was converted to the corresponding 2-amino-6,8-dichloropurine derivative in which the 6-chloro group could be selectively displaced by various nucleophiles[69]. 8-Fluoroadenosine was synthesized via a modified application of the Schiemann reaction on tri-O-acetyl-8-aminoadenosine[70]. Replacement of the 6-amino group of adenosine with fluorine could be achieved by reaction of its trimethylammonium salt with KF in butanol[71]. Several N^4-substituted derivatives of 1-β-D-arabinofuranosylcytosine (ara-C) and 5-fluoro-ara-C were prepared from the corresponding uracil arabinoside via thiation, S-methylation, and nucleophilic displacement of the 4-thiol (or methylthio) group with NH_2OH, NH_2, and CH_3NH_2[72].

The previously reviewed (1967) interesting transformations of 5-halo-arabinosyluracils into imidazoline nucleosides on heating in aqueous media have been studied in more detail, and the products were compared with imidazoline nucleosides obtained by direct synthesis[73]. Further studies revealed that also 5-halouridines[74] and 5-hydroxyuridines[75] undergo alkali-catalyzed ring contractions on heating to give imidazoline nucleosides. The transformations of the 5-halouridines were shown to require participation of the 5'-hydroxyl group of the sugar moiety (which, via nucleophilic attack at C_6, facilitates hydrolysis at C_5 of the pyrimidine ring)[74], and thus their mechanism appears to be analogous to that of the 5-haloarabinosyluracils (where the 2'-hydroxyl group participates)[73]. The 5-hydroxyuridines appear to undergo a benzilic acid-type rearrangement and dehydration not requiring anchimeric assistance by the sugar moiety; 1-methyl and 1,3-dialkyl-5-hydroxyuracils also undergo ring contractions on heating with aqueous alkali, to give the corresponding imidazoline derivatives[75]. Deuterium exchange studies support the suggestion that these reactions involve ine 5-keto tautomer of the 5-hydroxyuracil derivatives[75].

Intramolecular participation of the 2'-hydroxyl group appears to enhance the rate of the hydrolytic deamination of 1-β-D-arabinosylcytosine (ara-C[76]). Rate studies of base-catalyzed deuterium exchange[77,78] at C_5 or C_6 of various uracil nucleosides and other uracil derivatives demonstrated the anchimeric assistance of the (ionized) 2'-(arabo)-and 5'-hydroxyl groups of the glycosyl moieties in the exchange reaction at C_5[77] and indicated that the base catalysis may involve either 1,2-or 1,4 additions of the base to the double bond system[78]. Similar studies of the mechanism of reaction of N_1-substituted-5-bromouracils with sulfur nucleophiles has led to a new method for introduction of a 5-sulfur substituent into the pyrimidine ring of uracil nucleotides via a methyl hypobromite adduct[79]. An oxygen-catalyzed reaction between 4-thiouridine and sodium sulfite gave a product characterized as uridine-4-sulfonate which was converted quantitatively at pH4 into uridine or at pH 8.5 into cytidine[80]. The action of NH_2OH (a mutagen) on 2'-deoxycytidine appears to involve nucleophilic addition of the reagent to the double bond at C_6 followed by replacement of the 4-amino group by a second molecule of NH_2OH[81]. The 5,6-hypobromite adducts of uracil nucleosides on refluxing in neutral aqueous solutions are converted to the 5,6-dihydroxy-5,6-dihydro derivatives[82]. Several papers present extensive studies relating to the photolytic[83,84], reductive, or photoreductive[85,86] and oxidative[87] degradations of pyrimidine nucleosides involving the 5,6 double bond.

Syntheses of new 8,2'-O-anhydro[88] and 8,5'-O-anhydro[89] nucleosides of adenine and guanine, by cyclization of the appropriate 8-bromopurine nucleosides, were reported. Additional N-substituted-imino-bridged analogs[90] and a new sulfur-bridged analog[91] of 2,3'-anhydrothymidine were synthesized. A novel method was reported for the direct cyclization of 2'-deoxynucleosides to the 2,3'-anhydro derivatives with diethyl (2-chloro-1,1,2-trifluoroethyl)amine[92]. Arabino-and/or lyxofuranosyl derivatives of 6-azaisocytosine[93], 6-azauracil[94], isocytosine[95], and

cytosine[96] were prepared via anhydronucleoside intermediates or by direct sulfonyloxy group displacements. Reaction of a 3',5'-anhydronucleoside (epoxide) with lithium azide gave the 5'-azide[97]. A new synthesis of cytosine arabinoside by silylation of cytidine-2',3'-cyclic phosphate was reported[98].

Selective halogenation of nucleosides at the 5'-position was effected with (halomethylene) dimethylammonium halides, $[(CH_3)_2N = CHX]^+X^-$, derived from the reaction of dimethylformamide with phosgene, $SOCl_2$ or $SOBr_2$, or other halogenating agents ($POCl_3$,PCl_5)[99]. Other modifications of the 5'-hydroxymethylene group reported include oxidation of the 2',3'-isopropylidene derivative with a ketenimine and dimethyl sulfoxide to the 5'-aldehyde[100], and with $KMnO_4$ to the 5'-carboxylic acid[101]. 5'-O-Sulfamoyl nucleosides (desfluoro analogs of nucleocidin) were prepared[102]. Reactions leading to extension of the sugar chain were reported[103].

Naturally Occurring Nucleosides - The previously reviewed (1967) unequivocal structure proof and total syntheses of the pyrrolopyrimidine nucleoside antibiotics, toyocamycin, sangivamycin, and tubercidin were reported in more detail[104]. On the basis of ^1H and ^{19}F nmr and mass spectroscopy, the revised structure of the antitrypanosomal antibiotic nucleocidin was established as 9-(4-fluoro-5-O-sulfamoylpentofuranosyl) adenine, the first fluoro-sugar derivative isolated from natural sources[105,106]. The unsaturated branched-chain sugar nucleoside, angustmycin A (decoyinine)[107] and an amino-sugar nucleoside of cytosine identical with a product derived from gougerotin[108] were synthesized. New "minor" constituents isolated from tRNA include: N-(purinyl-6-carbamoyl)threonine[109], 2-methylthio-IPA[110], methyl 2-thiouridine-5-acetate[111], 5-carboxymethyluridine[112], 2-thiocytosine, and 5-methylaminomethyl-2-thiouracil[113].

Synthesis of Nucleotides, Phosphorylation and Protection - Selective phosphorylation of the 5'-OH of unprotected nucleosides was achieved with bis-[β,β,β-trichloroethyl] phosphorochloridate[114]. The trichloroethyl groups can be removed by treatment with zinc dust in pyridine or, alternatively with zinc-copper complex in dimethyl formamide, to give the free 5'-phosphates[114]. Pyrophosphoryl chloride, in various organic solvents (including m-cresol and o-chlorophenol), was also reported to phosphorylate various nucleosides selectively at the primary OH group[115]. A more complex reagent for the selective phosphorylation of the 5'-OH consists of an equimolar mixture of triphenylphosphine, dibenzyl hydrogen phosphate and diethylazodicarboxylate[116]. The new phosphorylating agent[117], pyridinium S-ethyl phosphorothioate with dicyclohexylcarbodiimide (DCC), (presumbly not selective) was used for the preparation of the nucleoside-5'S-ethyl phosphorothioates which can be converted to the 5'-phosphates by treatment with aqueous I_2[117], or to the 5'-triphosphates by treatment with I_2 and inorganic pyrophosphate[140]. In contrast, sodium trimetaphosphate was reported to effect specific phosphorylation of the 2'- and 3'-hydroxyls of unblocked ribonucleosides[118]. Selective reaction of triethyl

phosphite with the 2' (3') -hydroxyl of unblocked nucleosides followed by oxidation of the resulting 2' (3') -phosphite with hexachloroacetone leads directly to the 2' , 3' -cyclic phosphates; this procedure appears to provide a convenient general method of synthesis for the cyclic phosphates of nucleosides having a cis-1,2 diol system[119]. The large number of new mononucleotides synthesized via phosphorylation, or by transformation of preformed nucleotides, cannot be reviewed here, and only the phosphate analogs, 5' -deoxynucleoside 5' -methylenephosphonates[120,121], 5' -S-phosphorothioates[122], and 5' -N-phosphoramidates[122,123] are included in the references. A review chapter on the chemistry of mononucleotides was published recently[124].

New reagents used for the introduction of selectively removable protecting groups at either the primary or secondary sugar hydroxyls of nucleosides and nucleotides include tris-(2-chloroethyl) orthoformate, (the bis-(2-chloroethyl) orthoformate ester of the nucleoside is base-stable but hydrolyzable with 80% acetic acid at room temperature)[125], β,β,β-tribromoethyl chloroformate (the tribromoethoxycarbonate ester of the nucleoside is acid-stable, but can be cleaved with a zinc-copper complex)[126], dihydrocinnamoyl chloride or anhydride (the ester formed can be hydrolyzed enzymatically with α-chymotrypsin)[127], benzoylformyl chloride (the benzoylformyl group can be selectively removed by hydrolysis with aqueous pyridine at room temperature)[128], 2,4-dinitrobenzenesulfenyl chloride (the ester formed is selectively cleaved by thiophenol in pyridine)[129], and β-benzoylpropionic acid with DCC (the β-benzoylpropionyl group can be selectively removed with dilute hydrazine hydrate solution in pyridine or acetic acid)[128]. A new reagent used for specific blocking of the cis-2' ,3' -diol system is tetramethyl orthocarbonate; this reagent, in the presence of p-toluenesulfonic acid in dioxane, converts ribonucleosides into 2' , 3' -O-dimethoxymethylidene derivatives which are base-stable but which can be transformed with aqueous acid quantitatively into the base-labile 2' ,3' -cyclic carbonates[130].

For protection of primary NH_2 groups in the heterocyclic moieties of cytosine, adenine, and guanine nucleotides, the following N-blocking groups have been used recently: dimethylaminomethylene (readily introduced by reaction of the nucleotides with dimethylformamide acetals[131], and removed with either weak acid or base[132]), isobutyloxycarbonyl (introduced on N^4 of cytosine nucleotides by reaction with isobutyl chloroformate followed by treatment with NaOH in dioxane-water; removed by concentrated NH_4OH)[128], and the benzoyl group which was found to be selectively removable from the NH_2 groups by treatment with 0.5 M hydrazine hydrate in pyridine-acetic acid, without cleavage of the commonly used OH- and phosphate-protecting groups[133].

As phosphate-protecting groups, in addition to the β -cyanoethyl, the β,β,β-trichloroethyl[114] and ethylthio[119] ester and aromatic amidate[141] groups appear to be the most promising. The nucleoside β,β,β-trichloroethyl phosphates[114] and S-ethyl phosphorothioates[119] are prepared directly from the unblocked or partially

blocked nucleosides, respectively (see above); they are (in contrast to the β-cyanoethyl phosphates) stable under the alkaline conditions used for the removal of the conventional O- and N-blocking groups, and are, in turn, deprotected by the specific procedures already mentioned. The aromatic amidate (p-anisidate) blocking group is introduced by treating the nucleotide with the aromatic amine in the presence of DCC, and it is removed with isoamyl nitrite[141].

Synthesis of Oligo- and Polynucleotides - A large number of dinucleoside phosphates, di-, tri-, and polynucleotides was synthesized by chemical and/or enzymatic methods, including those containing "minor" bases of tRNA, or unnatural base analogs. In addition, the internucleotide phosphodiester linkage was replaced with the non-ionic carbonate (diester)[134] and carboxymethyl (ester-ether)[135] linkages to give the corresponding dinucleotide analogs; a poly (3' -O-carboxymethylthmidine) polymer (M.W. >4000) was also prepared using dicyclohexylcarbodiimide (DCC) as condensing agent[135].

Further extensions of the β-cyanoethyl phosphotriester method (in which a β-cyanoethyl-"protected" 3' -phosphate end group is condensed without deprotection with the free 5' -OH of the next nucleoside unit, using mesitylenesulfonyl chloride as condensing agent)[136,137], as well as applications of the new β,β,β-trichloroethyl phosphates (either in an essentially similar phosphotriester approach[138] or, alternatively, as the protected 5' -phosphate end group in deoxyribo-oligonucleotide synthesis with DCC[139]) are described. Various potential applications of the S-ethyl phosphorothionates in deoxyribo-oligonucleotide synthesis (either as protecting groups in condensations with DCC or, alternatively, as activated groups in the presence of I_2-pyridine, undergoing phosphodiester bond formation without a condensing agent) have been studied[140]. An attractive new method for the stepwise synthesis of protected ribooligonucleotides with a 3' -phosphate end group, applicable to large scale synthesis, utilized the p-anisidate group for the protection of each added (partially blocked) nucleoside-3' -phosphate unit; the p-anisidate group is selectively removed from the new terminal 3' -phosphate after each condensation step[141]. In a synthesis of a deoxyribo-oligonucleotide, the unusual phosphate-protecting group 2',3' -[2,4-dimethoxybenzylidene] uridine was employed which could be removed by periodate oxidation of the cis-1,2 diol present only in the uridine moiety. In an interesting study, appropriate protecting groups were introduced into unprotected dinucleoside phosphates, for the purpose of making RNA-fragments usable as starting materials for oligonucleotide synthesis[143].

Pertinent to the overall strategy of polynucleotide synthesis, the use of preformed oligonucleotide blocks was further investigated[144], and a new method termed "fragment coupling" was introduced, which involves the condensation of suitably protected oligonucleotides having 5' -phosphomonoester end groups, in such manner that the chain length should be approximately doubled at each condensation step[145]. Further studies aimed at the development of practical procedures for the synthesis of oligonucleotides on polymer supports have been continued[146-148], and a method for

polymer-supported sequential analysis of polyribonucleotides has been published.[149]

REFERENCES

1. "Synthetic Procedures in Nucleic Acid Chemistry" by W. W. Zorbach and R. S. Tipson (Eds.), Vol. I., J. Wiley and Sons, Inc., New York (1968).
2. E. J. Reist, L. V. Fisher, and L. Goodman, J. Org. Chem. 33, 189 (1968).
3. K. J. Ryan, E. M. Acton, and L. Goodman, J. Org. Chem., 33, 1783 (1968).
4. M. L. Wolfrom and M. W. Winkley, J. Org. Chem., 33, 4227 (1968).
5. R. F. Nutt, M. J. Dickinson, F. W. Holly, and E. Walton, J. Org. Chem., 33, 1789 (1968).
6. S. R. Jenkins, B. Arison, and E. Walton, J. Org. Chem., 33, 2490 (1968).
7. A. Rosenthal and L. B. Nguyen, J. Org. Chem., 34, 1029 (1969).
8. E. J. Reist, D. F. Calkins, and L. Goodman, J. Amer. Chem. Soc., 90, 3852 (1968).
9. A. Rosenthal, M. Sprinzl, and H. J. Koch, Can. J. Chem., 47, 3263 (1969).
10. A. Rosenthal and M. Sprinzl, Can. J. Chem., 47, 3941; 4477 (1969).
11. R. F. Nutt and E. Walton, J. Med. Chem., 11, 151 (1968).
12. L. M. Lerner, J. Org. Chem., 34, 101 (1969).
13. L. M. Lerner, B. D. Kohn, and P. Kohn. J. Org. Chem., 33, 1780 (1968).
14. I. M. Downie, J. B. Lee, T. J. Nolan, and R. M. Allen, Tetrahedron Lett., 1969, 2339.
15. G. T. Rogers and T. L. V. Ulbricht, Tetrahedron Lett., 1968, 1025.
16. G. T. Rogers and T. L. V. Ulbricht, J. Chem. Soc., 1968, 1929.
17. J. A. Montgomery and H. J. Thomas, J. Org. Chem., 34, 2647 (1969).
18. J. D. Stevens, R. K. Ness, and H. G. Fletcher, Jr., J. Org. Chem., 33, 1806 (1968).
19. M. Haga, R. K. Ness, H. G. Fletcher, Jr., J. Org. Chem. 33, 1810 (1968).
20. N. J. Leonard and M. Rasmussen, J. Org. Chem. 33, 2488 (1968).
21. H. Iwamura and T. Hashizume, J. Org. Chem., 33, 1796 (1968).
22. J. A. Montgomery and K. Hewson, J. Med. Chem., 11, 48 (1968).
23. M. J. Robins and R. K. Robins, J. Org. Chem., 34, 2160 (1969).
24. Y. Furukawa and M. Honjo, Chem. Pharm. Bull. (Tokyo), 16, 1076 (1968).
25. B. Shimizu and M. Miyaki, Tetrahedron Lett., 1968, 855.
26. E. Walton, F. W. Holly, and S. R. Jenkins, J. Org. Chem., 33, 192 (1968).
27. Y. Fulmer Shealy and A. O'Dell, Tetrahedron Lett., 1969, 2231.
28. N. J. Leonard, F. C. Sciavolino, V. Nair, J. Org. Chem., 33, 3169 (1968).
29. R. Vince and J. Conovan, J. Med. Chem., 12, 175 (1969).
30. M. Prystas and F. Sorm, Coll. Czech. Chem. Commun., 34, 2316 (1969).
31. H. -J. Lee and P. W. Wigler, Biochemistry, 7, 1427 (1968).
32. T. B. Øyen, Biochem. Biophys. Acta, 186, 237 (1969).
33. P. W. Wigler and H. -J. Lee, Biochemistry 8, 1344 (1969).
34. M. Prystas and F. Sorm, Coll. Czech. Chem. Commun., 33, 210 (1968).

35. G. T. Rogers, R. S. Shadbolt, and T. L. V. Ulbricht, J. Chem. Soc., C, 1969, 209.
36. K. A. Watanabe and J. J. Fox, J. Heterocyclic Chem., 6, 109 (1969).
37. G. T. Rogers and T. L. V. Ulbricht, Chem. Comm., 1969, 508.
38. T. Ueda and H. Nishimu, J. Amer. Chem. Soc., 90, 1678 (1968).
39. E. Wittenburg, Chem. Ber., 101, 1095; 1614; 2132 (1968).
40. M. W. Winkley and R. K. Robins, J. Org. Chem., 33, 2822 (1968).
41. M. W. Winkley and R. K. Robins, J. Org. Chem., 34, 431 (1969).
42. H. Vorbruggen, P. Strehlke, and G. Schulz, Angew. Chem. Int. Ed., 8, 976 (1969).
43. A. F. Wu and E. Chargaff, Proc. Natl. Acad. Sci., 63, 1222 (1969).
44. M. P. Kotick, C. Szantay, and T. J. Bardos, J. Org. Chem., 34, 3806 (1969).
45. M. Prystas and F. Sorm, Coll. Czech. Chem. Commun., 34, 1104 (1969).
46. M. J. Robins and B. L. Currie, Chem. Commun., 1968, 1597.
47. M. G. Stout and R. K. Robins, J. Org. Chem., 33, 1219 (1968).
48. M. J. Covill, H. G. Garg, and T. L. V. Ulbricht, Tetrahedron Lett., 1968, 1033.
49. G. Szekeres and T. J. Bardos, Amer. Chem. Soc., 158th Meeting, Sept. 1969, New York, N. Y., Abstracts, MEDI-69; J. Med. Chem., 13, in press.
50. A. Holy and F. Sorm, Coll, Czech. Commun. 34, 3383 (1969).
51. D. M. Brown, M. G. Burdon, and R. P. Slatcher, J. Chem. Soc., C, 1968, 1051.
52. M. Bobek and J. Farkas, Coll. Czech. Chem. Commun. 34, 1684, 1684 (1969).
53. R. J. Rousseau, R. K. Robins, and L. B. Townsend, J. Amer. Chem. Soc., 90, 2661 (1968).
54. R. J. Rousseau and L. Townsend, J. Org. Chem., 33, 2828 (1968).
55. J. A. Montgomery and H. J. Thomas, Chem. Commun., 1969, 458.
56. M. Bobek, J. Farkas, and F. Sorm, Tetrahedron Lett., 1968, 1543.
57. R. L. Tolman and L. B. Townsend, Tetrahedron Lett., 1968, 4815.
58. M. H. Fleysher, A. Bloch, M. T. Hakala, and C. A. Nichol, J. Med. Chem., 12, 1056 (1969).
59. G. Shaw, B. M. Smallwood, and D. V. Wilson, J. Chem. Soc. C, 1968, 2999.
60. D. M. G. Martin and C. B. Reese, J. Chem. Soc., C, 1968, 1731.
61. N. J. Leonard, S. M. Hecht, F. Skoog, and R. Y. Schmitz, Proc. Natl. Acad. Sci., 59, 15 (1968).
62. G. B. Chheda and R. H. Hall, J. Org. Chem., 34, 3492 (1969).
63. G. B. Chheda, R. H. Hall, and P. M. Tanna, J. Org. Chem., 34, 3498 (1969).
64. A. D. Broom and R. K. Robins, J. Org. Chem., 34, 1025 (1969).
65. Y. Kanaoka, E. Sato, M. Aiura, O. Yonemitsu, and Y. Mizuno, Tetrahedron Lett., 1969, 3361.
66. A. P. Martinez and W. W. Lee, J. Org. Chem., 34, 416 (1969).
67. A. Giner-Sorolla, C. Nanos, J. H. Burchenal, M. Dollinger, and A. Bendich, J. Med. Chem., 11, 52 (1968).

68. E. J. Reist, D. F. Calkins, L. V. Fischer, and L. Goodman, J. Org. Chem., 33, 1600 (1968).
69. J. F. Gerster, B. C. Hinshaw, R. K. Robins, and L. B. Townsend, J. Org. Chem., 33, 1070 (1968).
70. M. Ikehara and S. Yamada, Chem. Commun., 1968, 1509.
71. J. Kiburis and J. H. Lister, Chem. Commun., 1969, 381.
72. I. Wempen, N. Miller, E. A. Falco, and J. J. Fox, J. Med. Chem., 11, 144 (1968).
73. B. A. Otter, E. A. Falco, and J. J. Fox, J. Org. Chem., 33, 3593 (1968).
74. B. A. Otter, E. A. Falco, and J. J. Fox, J. Org. Chem., 34, 1390 (1969).
75. B. A. Otter, E. A. Falco, and J. J. Fox, J. Org. Chem., 34, 2636 (1969).
76. R. E. Notari, M. L. Chin, and A. Cardoni, Tetrahedron Lett., 1969, 3499.
77. D. V. Santi, and C. F. Brewer, J. Amer. Chem., Soc., 90, 6236 (1968).
78. R. J. Cushley, S. R. Lipsky, and J. J. Fox, Tetrahedron Lett., 1968, 5393.
79. L. Szabo, T. I. Kalman, and T. J. Bardos, Amer. Chem. Soc., 158th Meeting, Sept. 1969, New York, N. Y. Abstracts p. MEDI-72; J. Org. Chem., 35, in press.
80. H. Hayatsu, J. Amer. Chem. Soc., 91, 5693 (1969).
81. D. M. Brown and M. J. E. Hewlins, J. Chem. Soc., C, 1968, 1922.
82. T. Kanai, M. Ichino, and T. Kojima, Chem. Pharm. Bull. (Tokyo), 17, 650 (1969).
83. Y. Kondo and B. Witkop, J. Amer. Chem. Soc., 90, 3258 (1968).
84. N. Miller and P. Cerutti, Proc. Nat'l. Acad. Sci., 59, 34 (1968).
85. Y. Kondo and B. Witkop, J. Amer. Chem. Soc., 90, 764 (1968).
86. P. Cerutti, Y. Kondo, W. R. Landis, and B. Witkop, J. Amer. Chem. Soc., 90, 771 (1968).
87. P. Howgate, A. S. Jones, and J. R. Tittensor, J. Chem. Soc., C, 1968, 275.
88. M. Ikehara, H. Toda, and M. Kaneko, Tetrahedron Lett., 1968, 3489.
89. M. Ikehara and M. Kaneko, J. Amer. Chem. Soc., 90, 497 (1968).
90. I. L. Doerr, R. J. Cushley, and J. J. Fox, J. Org. Chem., 33, 1592 (1968).
91. L. Wempen and J. J. Fox, J. Org. Chem., 34, 1020 (1969).
92. G. Kowollik, K. Gaertner, and P. Langen, Tetrahedron Lett., 1969, 3863.
93. J. Beranek and F. Sorm, Coll. Czech. Chem. Commun., 33, 913 (1968).
94. J. Beranek, Coll, Czech. Chem. Commun., 34, 618 (1969).
95. M. Hirata, Chem. Pharm. Bull, (Tokyo), 16, 430 (1968).
96. T. Kanai and M. Ichino, Chem. Pharm. Bull., (Tokyo), 16, 1848 (1968).
97. K. Kikugawa, M. Ichino, T. Kusama, and T. Ukita, Chem. Pharm. Bull., (Tokyo), 17, 798 (1968).
98. J. Nagyvary, J. Amer. Chem. Soc., 91, 5409 (1969).
99. R. F. Dods and J. S. Roth, Tetrahedron Lett., 1969, 165.
100. R. E. Harmon, C. V. Zenarosa, and S. K. Gupta, Chem. Commun., 1969, 327.
101. R.R. Schmidt, U. Scholcz, and D. Schwille, Chem. Ber., 101, 590 (1968).

102. D.A. Shuman, R.K. Robins, and M.J. Robins, J. Amer. Chem. Soc., 91, 3391 (1969).
103. G. Etzold, G. Kowollik, and P. Langen, Chem. Commun., 1968, 422.
104. R.L. Tolman, R.K. Robins, and L.B. Townsend, J. Amer. Chem. Soc., 91, 2102 (1969).
105. L. Fowden, Proc. Royal Soc. (London), B171, 5 (1968).
106. G.O. Morton, J.E. Lancaster, G.E. Van Lear, W. Fulmor, and W.E. Meyer, J. Amer. Chem. Soc., 91, 1535 (1969).
107. J.R. McCarthy, Jr., R.K. Robins, and M.J. Robins, J. Amer. Chem. Soc., 90, 4993 (1968).
108. K. Watanabe, M.P. Kotick, and J.J. Fox, Chem. Pharm. Bull., (Tokyo), 17, 416 (1969).
109. M.P. Schweizer, G.B. Chheda, L. Baczynskyj, and R.H. Hall, Biochemistry, 8, 3283 (1969).
110. W.J. Burrows, D.J. Armstrong, F. Skoog, S.M. Hecht, J.T. Boyle, N.J. Leonard, and J. Occolowitz, Science, 161, 691 (1968).
111. L. Baczynskyj, K. Biemann, and R.H. Hall, Science, 159, 1481 (1968).
112. M.W. Gray and B.G. Lane, Biochemistry, 7, 3441 (1968).
113. J. Carbon, H. David, and M.H. Studier, Science, 161, 1146 (1968).
114. A. Franke, K.H. Scheit, and F. Eckstein, Chem. Ber., 101, 2998 (1968).
115. K.I. Imai, S. Fujii, K. Takanohashi, Y. Furukawa, T. Masuda, and M. Honjo, J. Org. Chem., 34, 1547 (1969).
116. O. Mitsunobu, K. Kato, and J. Kimura, J. Amer. Chem. Soc., 91, 6510 (1969).
117. A.F. Cook, M.J. Holman, and A.L. Nussbaum, J. Amer. Chem. Soc., 91 1522 (1969).
118. A.W. Schwartz, Chem. Commun., 1969, 1393.
119. A. Holý, Coll. Czech. Chem. Commun., 33, 2245 (1968); 34, 3510 (1969).
120. J. Montgomery and K. Hewson, Chem. Commun., 1969, 15.
121. G.H. Jones and J.G. Moffatt, J. Amer. Chem. Soc., 90, 5337 (1968).
122. A. Hampton, L.W. Brox, and M. Bayer, Biochemistry, 8, 2303 (1969).
123. B. Jastorff, H. Hettler, Chem. Ber., 102, 4119 (1969).
124. T. Ueda and J.J. Fox, Adv. Carbohydrate Chem., 22, 307 (1967).
125. T. Hata and J. Azizian, Tetrahedron Lett., 1969, 4443.
126. A.F. Cook. J. Org. Chem., 33, 3589 (1968).
127. H.S. Sachdev and N.A. Starkovsky, Tetrahedron Lett., 1969, 733.
128. R.L. Letsinger and P.S. Miller, J. Amer. Chem. Soc., 91, 3356 (1969).
129. G.W. Grams and R.L. Letsinger, J. Org. Chem., 33, 2589 (1968).
130. G.R. Niaz and C.B. Reese, Chem. Commun., 1969, 552.
131. A. Holý, S. Chladek, and J. Zemlicka, Coll. Czech. Chem. Commun., 34, 253 (1969).
132. A. Holy and J. Zemlicka, Coll. Czech, Chem. Commun., 34, 2449 (1969).
133. R.L. Letsinger, P.S. Miller, and G.W. Grams, Tetrahedron Lett., 1968, 2621.
134. M.P. Mertes and E.A. Coats, J. Med. Chem., 12, 154 (1969).
135. M.H. Halford and A.S. Jones, Nature 217, 638 (1968).

136. R.L. Letsinger and K.K. Ogilvie, J. Amer. Chem. Soc., 91, 3351 (1969).
137. R.L. Letsinger, K.K. Ogilvie, and P.S. Miller , J. Amer. Chem. Soc., 91, 3360 (1969).
138. F. Eckstein and I. Rizk, Chem. Ber., 102, 2362 (1969).
139. A. Franke, F. Eckstein, K.-H. Scheit, and F. Cramer, Chem. Ber., 101, 944 (1968).
140. A.F. Cook, M.J. Holman, and A.L. Nussbaum, J. Amer.Chem. Soc., 91, 6497 (1969).
141. E. Ohtsuka, K. Murao, M. Ubasawa, and M. Ikehara, J. Amer. Chem. Soc., 91, 1537 (1969).
142. F. Kathawala and F. Cramer, Liebigs Ann. Chem., 712, 195 (1968).
143. J. Smrt, Coll. Czech. Chem. Commun., 33, 1462 (1968).
144. J. Hachmann and H.G. Khorana, J. Amer. Chem. Soc., 91, 2749 (1969).
145. S.A. Narang, S.K. Dheer, and J.J. Michniewicz, J. Amer. Chem. Soc., 90, 2702 (1968).
146. L.R. Melby and D.R. Strobach, J. Org. Chem., 34, 421; 427 (1969).
147. T. Shimidzu and R.L. Letsinger, J. Org. Chem., 33, 708 (1968).
148. F. Cramer and H. Köster, Angew Chemie Int. Ed., 7, 473 (1968).
149. T.E. Wagner, H.G. Chai, and A.S. Warfield, J. Amer. Chem. Soc., 91, 2388 (1969).

Chapter 32. Antiradiation Agents

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Introduction - This report is based on articles selected from the 800 included in Parts 17-20 of the author's bibliography[1] covering 1968 and 1969. As in the three previous reports in this series no attempt has been made to cover all developments in radiobiology.

The period of this report was characterized by the maintenance of the same level of effort in the field that has been expended during most of the past ten years. There has been an increased effort to apply the extensive knowledge of the well-known aminothiol class to the solution of problems in other areas of medicine and to determine the mechanisms of radiation damage and of protection by these agents.

The WRAIR Program - In our 1967 report we summarized the extensive program sponsored by the Department of the Army in the Division of Medicinal Chemistry, Walter Reed Army Institute of Research. Unfortunately the publication of quantitative pharmacological data by WRAIR has been delayed. Data for specific compounds may be obtained from those participants who prepared them for evaluation by WRAIR. The data given to participants include LD_{50}'s and 30-day survival data (mouse test). Human tolerance studies for some compounds are planned.

Reviews - Two general reviews have appeared.[2,3] Specialized reviews are now available describing the pharmacological aspects of antiradiation drugs (particularly the aminothiols),[4,5,6,7] the rôle of thiol groups in determining natural radioresistance,[8] and the rôle of thiol groups in enzyme function and radioresistance.[9] The Masson series has continued.[10]

Drug Evaluation - The methods now used for the evaluation of antiradiation drugs have been summarized.[11] The complexities that arise in the use of whole animals as test systems are as prevalent here as in other areas of pharmacology. For example, the failure of some investigators to maintain an adequate supply of oxygen detracts from the validity of their work because of the radioprotective effect of anoxia.[12] The test system is an important a variable as the physical quality of the radiation used.[13] Earlier assertions that the radiosensitivity of rats depended on the time of day at which they were irradiated have now been disputed.[14] The radioresistance of hibernating ground squirrels, first reported over ten years ago, is now understood.[15,16]

In efforts to overcome these and other complexities drug evaluations are being made in simpler systems, although the extrapolation of the results of such work to cover protection of intact mammals is not exact.[17] A good correlation between the radioprotective potency of aminothiol drugs and their ability to protect the erythropoietic system of mice (as measured by ^{59}Fe uptake) has been obtained;[18] no correlation was observed when the serotonin type of radioprotector was used. The method can be used for

measuring protection against doses as low as 100 r.[19] An analogous evaluation of aminothiols has been made in tissue culture.[20]

Other evaluation technics described recently include the use of the mouse hair follicle,[21] enzymes *in vitro*,[22] spleen colony-forming rate,[23,24] and the fish O. latipes.[25]

Drug Development - During the period of this report there have been many publications of pharmacological data for the well-established antiradiation drugs such as MEA, AET and serotonin; the indexes to Chemical Abstracts should be consulted. Improved drug transport to, and selective absorption by, radiation sensitive tissues was sought by preparing 47 mercaptoethyl derivatives of drugs already known to be transported and selectively absorbed *in vivo*. None was found to give better protection than MEA.[26]

A series of 144 N-substituted S-2-aminoethyl thiosulfates (Bunte salts) was prepared.[27] Many members gave good protection to mice at doses of 0.07-0.1 of the LD_{50}. A comparison of the pharmacology of a series of Bunte salts with that of the related aminothiols[28] showed that the Bunte salts were less toxic to mice, but that the differences in radioprotective potency between members of each series could not be predicted accurately. The optimum spacing between the amino and thiosulfate groups was two carbon atoms. Quantitative data were given for protection by 12 of the compounds. In a parallel study[29] it was observed that the potency of 2-guanidinoethanethiosulfuric acid was decreased when the guanidino group was alkyl-substituted. Significant protection was obtained only when the nitrogen and sulfur functions were separated by two carbon atoms. α-Acetamidinium Bunte salts ($RNHC(NH)CH_2SSO_3H$), in which R was a terpenoid or adamantane group, were prepared.[30] When R=bornyl or *cis*-myrtanyl the compounds protected mice at 0.25-0.5 of the LD_{50}. An extensive review of organic thiosulfates was published.[31]

In the field of phosphorothioates work continued with the purely inorganic diammonium amidophosphorothioate.[32] In mice a dose reduction factor of about 1.08-1.15 times that of MEA was observed; the therapeutic index was 2.9 (MEA=1.3). The dose reduction factor was not increased by the concurrent administration of serotonin. S-[2-(3-Ethylaminopropylamino)ethyl]-dihydrogenphosphorothioate and the related 3-methylamino compound protected mice as effectively as the related compound having no substituent on the terminal nitrogen atom.[33] The latter compound protected the normal tissues of mice significantly more than it protected malignant tissues,[34] presumably because of the poor absorption of the phosphorothioate drugs in tumors, reported in 1967. A fate and distribution study[35] of $H_2NCH_2CH_2SP(O)(OH)_2$ (whose monosodium salt is "Cystaphos") showed that the compound was rapidly converted *in vivo* to MEA, which was then metabolized to taurine and sulfate. The desirable feature of the phosphorothioate class may be a masking of the toxicity of MEA and an improvement of its transport.

The dose reduction factor for dimethylsulfoxide (DMSO) absorbed through the tails of mice was found to be 1.35;[36] significant protection was provided to mice against 900 r by the vapors of DMSO when present during or

following irradiation.[37] Electron transfer from DMSO to cupric ion was observed and is believed to be the process involved in the protection of copper-containing enzymes by DMSO.[38]

Many newer drugs have been described during the period of this report; only those for which significant radioprotective action was reported can be mentioned here.

- α-Amidrazonium thiosulfates ($RNHNHC(NH)CH_2SSO_3H$).[39]
- S-2-Aminoethyl S'-3-carboxypropyl dithiocarbonate ($NH_2CH_2CH_2SCOSCH_2CH_2CH_2COOH$).[40]
- Thiocarbamoyl disulfides ($((CH_3)_2NC(S)SSC(CH_3)_3$ and $NH_2C(S)SSC(CH_3)_3$).[41]
- Sodium 4-(2-acetamidoethyldithio)butanesulfinate ($CH_3CONHCH_2CH_2SS(CH_2)_4SO_2Na$) and the related sulfonate.[42]
- The dithiocarbamate ester of 9-aminoacridine.[43]
- [3-(2-Mercaptoethylamino)propyl]oxamide ($NH_2COCONH(CH_2)_3NHCH_2CH_2SH$); 17 closely related compounds gave little or no protection.[44]
- 7,10-Ethano-1-thia-4,7-diazaspiro[4,5]decane, prepared by the condensation of 3-quinuclidinone with MEA.[45]
- Adrenochrome monoguanylhydrazone methanesulfonate ("S-adchnon"), said to have a dose reduction factor in mice of 1.32 at about 5 mg/kg.[46]
- Magnesium pemoline; some doses were given 2 weeks pre-irradiation.[47,48]
- Phenylhydrazine hydrochloride ($C_6H_5NHNH_2 \cdot HCl$) when administered 5-8 days before irradiation; stimulation of erythropoiesis and an increase in stem cells may explain the protective effect.[49]

Field and his associates have studied the use of stable hemimercaptals prepared by the reaction of MEA (and other medicinally important thiols) with trichloro- or trifluoroacetaldehyde. Those derived from MEA functioned as reservoir forms of MEA and protected mice.[50] In a continuing study,[51] mercaptals, mercaptoles, orthothioformates and thiazolidines have been used. Protection of mice by 2,2-dimethylthiazolidine,[51,52] and the presumed diethyl bis(2-aminoethylthio)malonate was reported.

The use of mixtures of drugs to obtain greater protection than can be obtained by single components has continued to make progress. Maisin and his collaborators[53,54] have used mixtures of well-known antiradiation drugs (MEA, AET, cysteine, reduced glutathione, serotonin creatinine sulfate) in mice to obtain dose reduction factors of 2.8, with decreased toxicity. Similar results were reported by others.[55,56] Combinations including PAPP and phosphorothioates have been used by Soviet workers[57,58] who also reported on fate and distribution of the drug components.[58] An extensive pharmacological study was made of the drug "Irradian" (or "Reducdyn"), whose active ingredients include cysteine and N-acetylhomocysteinethiolactone.[59]

Mechanisms of Damage and Protection - Investigations have continued in all the areas summarized in previous reports in this series, with a continuing increase in emphasis on fundamental reactions at the molecular level; these have been reviewed.[60,61]

It has been estimated that under anoxic conditions the portion of the indirect effect of radiation in E. coli B that can be abolished by freezing,

or by high concentrations of radical scavengers, contributes about half of the total lethal damage.[62] A correlation between the protective activity of sulfur-containing drugs and the rate constants[63,64] for their reaction with the radiolysis products of water was established.[65] A new "oxibase" parameter was developed for aminothiols.[66] This is an expression of electron density in the thiolate anion, equivalent to ease of oxidation. Superior drug structures were predicted.

The radiochemistry of the drugs themselves is of interest. Thiyl radicals formed by the radiolysis of cysteine, MEA, and the related disulfides in aqueous solution can build up a steady-state concentration and "scramble" many disulfide groups.[67] The radiolysis of aqueous mixtures of cystine and penicillamine disulfide gave a spectrum of products very similar to that obtained from the mixed disulfide of the two components. Cystine alone gave sulfonic acids, while penicillamine difulside, which is not radioprotective, gave none. Perhaps the ability to form sulfonic acids is characteristic of protective disulfides.[68]

An earlier mechanism for protection by AET involved its conversion in vivo to MEG and GED. A recent study[69] of ^{14}C- and ^{35}S-labelled AET in mice showed that neither MEG or GED was formed, but that AET was converted to 2-aminothiazoline, which may be a significant intermediate in the protective mechanism. It has been known since 1965 that there is a close correlation between the protective potency of certain thiazolines and that of the structurally-related aminothiols.

The protective action of tissue anoxia has been reviewed.[70] Because protection of mammals by serotonin, PAPP, etc. has been ascribed to their vasoconstrictive activity with consequent tissue anoxia, it was surprising to learn that the serotonin-creatinine sulfate complex protected both planaria[71] and bean sprouts,[72] where vasoconstriction cannot be a factor.

There has been a steady accumulation of evidence to support the idea that the most important damage to a cell that leads to mitotic failure is a macromolecular lesion of DNA in the nucleoprotein system.[73,74,75] Cellular radiosensitivity in 120 diverse organisms ranging from viruses to higher plants and animals was a direct function of the chromosome volume and nucleic acid content.[76] In the intact cell of bacteria, protection by thiols appears to be protection of a functional repair mechanism rather than prevention of DNA degradation,[77] but the need for more information about damage to DNA remains moot.

Electron spin resonance studies of irradiated DNA at 77°K permitted the detection of at least two distinct paramagnetic centers.[78] Pulse radiolysis technics have been used to study the reaction of DNA and related pyrimidine bases with water radiolysis products.[79,80,81] The free radicals produced in animal tissues have kinetic properties that are related to the radiosensitivity of those tissues.[82] In a continuing study of the repair of damaged macromolecules by destruction of free radical sites it was found that transfer of the unpaired electron to a thiol occurred best in acid solution, in which the thiol is undissociated.[83] Transfer to disulfide

drugs was independent of pH. When dry mixtures of nucleic acids and MEA were irradiated at 100°K it was observed that the MEA became bound to the phosphate portion of the nucleotide and that transfer of the unpaired electron to the sulfur atom occurred;[84] the observation clearly supports one of the older mechanisms suggested to explain protection by aminothiol drugs. MEA, AET and the like bind to DNA and affect it as crosslinking agents affect polymers in general; this observation may lead to a new type of radioprotective substance.[85] The protection of DNA by bound amino compounds could not be correlated with the protective potency of these compounds in vivo.[86] There are probably many reasons why such a correlation should fail; for example, the fundamental damage may not be to DNA, but to the non-histone proteins of the chromosomal sheath. A radiochemical study of lipoic acid suggested that a single free radical can denature such disulfide-containing proteins.[87]

The "biochemical shock" mechanism of protection, discussed in the 1966 Report, continue to be supported by new data.[88,89]

The use of radiation-induced free radical sites in tissues to initiate the polymerization of acrylamide revealed the presence of centers of damage in the microsomes and heavy mitochondria of rat cells.[90,91]

The important rôle of certain metal ions in the mechanisms of damage and protection was reviewed;[92] damaged metalloenzymes can no longer support normal electron transport processes in cells. While many amino acids form complexes with cupric ion, only those containing sulfur reduce it, and thus facilitate the regeneration of the damaged enzyme system. The sensitizing effect of oxygen was ascribed to its oxidation of cuprous ion; in this sense the protective sulfur-containing drugs simulate anoxia.[93] It was reported[94] that protective activity in a series of aminothiol and guanidinothiol drugs was parallel to the inhibition of catalase by these drugs. No such relationship was found for the inhibition of lactate dehydrogenase, where metal binding is unlikely. The drugs protected catalase by forming a complex with its iron component. The complexed catalase survived immediate radiation damage, and subsequent dissociation of the complex permitted normal decomposition of peroxides by the enzyme. The reference cited contains a good summary of evidence related to this mode of protection.

The protective effect of endogenous thiol groups continues to be of interest. The basic idea is that protein-bound glutathione represents a reservoir of protective material that can be released from the protein bond by exchange reactions with thiol[95] and other[96] drugs. Glutathione is known to be an excellent radical scavenger but is itself not useful as a drug because of its inability to penetrate cell walls. Administration of MEA, AET, and other thiol drugs caused a great increase in the intracellular nonprotein thiol level[97] and the protective activity of the drugs was correlated with this factor. At the time of maximum protection the MEA content of the cell was not over 10% of the sulfur compounds accumulating in the cell up to that time.[98] A xanthine oxidase inhibitor markedly increased the protective effect of cysteine presumably because it decreased the oxidation of glutathione liberated in the cells by the cysteine.[99]

The importance of endogenous thiols in the development of natural radioresistance is shown by the close correlation between the radioresistance of mice and the concentration of thiol in their blood-forming tissues of spleen homogenates. The great radioresistance of M. radiodurans is attributed to an abundant and expendable thiol component that protects the cell as a whole or protects its repair systems.[100] In a continuing study with Chinese hamster cells[101] it was shown that available intracellular thiol concentration is an important protective factor.

The case for endogenous thiols is not entirely clear-cut. The protective action for E. coli of cysteine was not correlated with an enhanced endogenous thiol concentration,[102] and no relation was found between endogenous nonprotein thiols and the radiosensitivity of HeLa, Ehrlich ascites tumor, and Chinese hamster cells.[103] The latter authors cannot ascribe the radiosensitizing effect of N-ethylmaleimide to a simple removal of endogenous thiols. The protective effect of prominent thiol drugs has been attributed to their causing an increased serotonin level, which is protective.[104]

Newer Uses for Aminothiols - Radioprotective aminothiols, particularly those that have higher therapeutic indexes and those that lack the convulsant activity shown by the simple aminothiols, have a potential application in the protection of normal cells during the radiotherapy of cancer.[105] Other uses are to promote wound healing after surgery of irradiated animals[106] and for the treatment of irreversible hemorrhagic shock.[107] One hears of still other uses (some quite exotic) but descriptions of these have not appeared in the open literature.

In the 1967 Report a short final paragraph referred to the possible use of prophylactic doses of antiradiation drugs to prevent ageing in cells, organs, and entire organisms. During the past two years Comfort has continued to stress the need for an experimental attack on the mechanisms of ageing and of methods for preventing it.[108] Experiments carried out with rodents and other short-lived animals should now be carried out in man. The life-shortening effects of acute and chronic exposures to radiation on experimental animals are well-known,[109,110] and are ascribed to free radical-induced abnormal cellular events. The earlier recommendations of Comfort and others that common food antioxidants (such as butylated hydroxytoluene) and aminothiols be used in prophylactic doses are under study; a review of early work is available.[111]

References

1. E. R. Atkinson, "Antiradiation Drugs - A Bibliography," Parts 17-20 (1968-1969). Available from the author at Arthur D. Little, Inc., Cambridge, Mass. 02140, or from Division of Medicinal Chemistry, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C. 20012.
2. W. O. Foye, J. Pharm. Sci., 58, 283 (1969); 364 refs.
3. E. F. Romantsev, "Radiation and Chemical Protection," Atomizdat, Moscow, 1968, 248 pp. (in Russian).

4. S. Akaboshi, Radioisotopes, 18, 60 (1969); Chem. Abstr., 71, 48245 (1969); 92 refs.
5. T. Sugahara, ibid., 18, 105 (1969); Chem Abstr., 71, 46400 (1969); 57 refs.
6. C. Liebecq, Rev. Med. Liege, 23, Suppl. 1, 129 (1968); Chem. Abstr., 71, 10043 (1969); 12 refs.
7. R. Goutier, ibid., 23, Suppl. 1, 135 (1968); Chem. Abstr., 71, 27763 (1969); 22 refs.
8. E. Y. Graevskii, Radiobiologiya, 7, 715 (1967); Chem. Abstr., 68, 19285 (1968); 161 refs.
9. T. Sanner, Scand. J. Clin. Lab. Invest., Suppl., 22, 53 (1968); Chem. Abstr., 71, 566 (1969).
10. (a) P. Ausloos, et al., "Actions Chimiques et Biologiques des Radiations," Masson, Paris, 1967, Series 11, 244 pp.
(b) M. Haissinsky (Ed.), ibid., 1968, Series 12, 340 pp.; reviewed in Science, 162, 1261 (1968).
11. P. S. Timaras and A. Vernadakis in A. Burger (Ed.), "Selected Pharmacological Testing Methods," Dekker, New York, 1968, pp. 307-336; 350 refs.
12. J. Schubert, Int. J. Radiat. Biol., 13, 297 (1967); Chem. Abstr., 68, 111981 (1968).
13. D. K. Bewley, Radiat. Res., 34, 446 (1968).
14. U. Reincke, Z. Naturforsch., 23b, 393 (1968).
15. R. E. Barr and X. J. Musacchia, Radiat. Res., 38, 437 (1969).
16. B. N. Jaroslow, D. E. Smith, M. Williams, and S. A. Tyler, ibid., 38, 379 (1969).
17. Z. M. Bacq, Strahlenschutz Forsch. Prax., 6, 49 (1966); Chem. Abstr., 69, 64973 (1968).
18. P. V. Vittorio, S. Dziubalo-Blehm and E. A. Aney, Radiat. Res., 37, 653 (1969).
19. R. Koch, Strahlenschutz Forsch. Prax., 6, 91 (1966); Chem. Abstr., 69, 64995 (1968).
20. A. J. Vergroesen, L. Budke and O. Vos, Int. J. Radiat. Biol., 13, 77 (1967); Chem. Abstr., 68, 36466 (1968).
21. T. E. Burlin, I. A. Magnus and L. Szur, Radiat. Res., 34, 287 (1968).
22. G. S. Melville and J. A. Winstead, ibid., 35, 547 (1968).
23. I. Feher, J. Gidali and L. Sztanyik, Int. J. Radiat. Biol., 14, 257 (1968), Chem. Abstr., 70, 17408 (1969).
24. E. J. Ainsworth and R. M. Larsen, Radiat. Res., 40, 149 (1969).
25. N. Egami, Int. J. Radiat. Biol., 15, 393 (1969); Chem. Abstr., 71, 109593 (1969).
26. R. D. Westland, J. L. Holmes, B. Green, and J. R. Dice, J. Med. Chem., 11, 824 (1968).
27. R. D. Westland, J. L. Holmes, M. L. Mouk, D. D. Marsh, R. A. Cooley, and J. R. Dice, ibid., 11, 1190 (1968).
28. D. L. Klayman, M. M. Grenan and D. P. Jacobus, ibid., 12, 510 (1969).
29. D. L. Klayman, M. M. Grenan and D. P. Jacobus, ibid., 12, 723 (1969).
30. J. M. Barton and L. Bauer, Can. J. Chem., 47, 1233 (1969).
31. D. L. Klayman and R. J. Shine, Quart. Rep. Sulfur Chem., 3, 191 (1968).
32. S. Åkerfeldt, C. Rönnbäck, M. Hellstrom, and A. Nelson, Radiat. Res., 35, 61 (1968).
33. J. R. Piper, C. R. Stringfellow and T. P. Johnston, J. Med. Chem., 12, 244 (1969).

34. J. M. Yuhas and J. B. Storer, J. Nat. Cancer Inst., 42, 331 (1969); Chem. Abstr., 70, 84777 (1969).
35. B. Shapiro and G. Kollman, Radiat. Res. 39, 552 (1969).
36. W. S. Moos and S. E. Kim, Experientia, 24, 450 (1968).
37. H. LeVan and V. Thanh-Huong, ibid., 24, 712 (1968).
38. W. Lohmann, Radiobiol. Biol. Mol., 1968, 1; Chem. Abstr., 70, 712 (1969).
39. T. L. Fridinger and J. E. Robertson, J. Med. Chem., 12, 1114 (1969).
40. T. P. Johnston, C. R. Stringfellow, and J. R. Piper, ibid., 11, 1256 (1968).
41. L. Field and J. D. Buckman, J. Org. Chem., 33, 3865 (1968).
42. L. Field and R. B. Barbee, ibid., 34, 1792 (1969).
43. W. O. Foye, D. H. Kay, and P. R. Amin, J. Pharm. Sci., 57, 1793 (1968); Chem. Abstr., 70, 11545 (1969).
44. R. D. Elliott and T. P. Johnston, J. Med. Chem., 12, 507 (1969).
45. W. Shapiro, M. F. Tansy, and S. Elkin, J. Pharm. Sci., 57, 1725 (1968); Chem. Abstr., 70, 11548 (1969).
46. T. Sugahara and T. Tanaka, Nature, 220, 271 (1968).
47. H. LeVan, Experientia, 24, 477 (1968).
48. H. LeVan and P. Burlakow, ibid., 25, 973 (1969).
49. L. H. Smith and T. W. McKinley, Radiat. Res., 39, 557 (1969).
50. L. Field, B. J. Sweetman, and B. Bellas, J. Med. Chem., 12, 624 (1969).
51. B. J. Sweetman, M. Bellas, and L. Field, ibid., 12, 888 (1969).
52. R. Granger, H. Orzalesi, and Y. Robbe, Trav. Soc. Pharm. Montpellier, 27, 23 (1967); Chem. Abstr., 68, 29643 (1968).
53. J. R. Maisin, Experientia, 24, 230 (1968).
54. J. R. Maisin, G. Mattelin, A. Fridman-Manduzio, and J. van der Parren, Radiat. Res., 35, 26 (1968).
55. R. I. H. Wang and A. T. Hasegawa, ibid., 40, 310 (1969).
56. E. J. Stork, A. E. Gass, and G. S. Melville, ibid., 35, 548 (1968).
57. L. F. Semenov, Zh. Vses, Khim. Obshchest., 14, 237 (1969); Chem. Abstr., 71, 19367 (1969).
58. G. M. Airapetyan, S. P. Yarmonenko, P. P. Lyarskii, V. G. Ovakimov, and V. V. Ivanov, Radiobiologiya, 9, 416 (1969); Chem. Abstr., 71, 77948 (1969).
59. E. Szirmai, B. Berkada, S. Hajdukovic, and j. M. Lopez-Gonzalez, Arzneimittel-Forsch., 18, 625 (1968).
60. A. N. Kuzin, "Molecular Mechanisms of Biological Effects Induced by High Energy Radiation," Nauka, Moscow, 1968, 311 pp.
61. B. N. Tarusov, Radiobiologiya, 7, 670 (1967); Chem. Abstr., 68, 19281 (1968); 32 refs.
62. T. Sanner and A. Pihl, Radiat. Res., 37, 216 (1969).
63. W. Karmann, A. Granzow, G. Meissner, and A. Henglein, Int. J. Radiat. Phys. Chem., 1, 395 (1969); Chem. Abstr., 71, 86544 (1969).
64. G. E. Adams, G. S. McNaughton, and B. D. Michael, Trans. Far. Soc., 64, 902 (1968).
65. O. H. Wheeler and R. A. Ribot, Chem. Ind. (London), 1969, 1017; Chem. Abstr., 71, 98771 (1969).
66. R. E. Davis, S. P. Molnar and R. Nehring, J. Amer. Chem. Soc., 91, 97 (1969).
67. T. C. Owen, M. Rodriguez, B. G. Johnson, and J. A. G. Roach, ibid., 90, 196 (1968).

68. J. W. Purdie, Can. J. Chem., 47, 1037 (1969).
69. A. G. Tarasenko, E. Y. Graevskii, M. M. Konstantinova, A. A. Mandrugin, I. V. Nekrasova, and V. M. Fedoseev, Dokl. Akad. Nauk SSSR, 187, 203 (1969); Chem. Abstr., 71, 77922 (1969).
70. L. K. Eidus, Radiobiologiya, 7, 678 (1968); Chem. Abstr., 68, 19282 (1968).
71. A. Laguarda-Figueras and R. Villalobos-Pietrini, Proc. Soc. Exp. Biol. Med., 126, 667 (1967); Chem. Abstr., 68, 66198 (1968).
72. R. Villalobos-Pietrini and A. Laguarda-Figueras, Radiat. Bot., 7, 369 (1967); Chem. Abstr., 68, 703 (1968).
73. J. S. Mitchell, Radiat. Chem. Aqueous Syst., Proc. L. Farkas Mem. Symp., 19th, 1967, 287 (1968); Chem. Abstr., 71, 88173 (1969).
74. E. Fahr, Angew. Chem. Internat. Ed., 8, 578 (1969); 137 refs.
75. K. F. Swingle and L. J. Cole, Curr. Top. Radiat. Res., 4, 189 (1968); Chem. Abstr., 68, 102358 (1968).
76. A, G. Underbrink and A. H. Sparrow, Radiat. Bot., 8, 205 (1968); Chem. Abstr., 69, 33401 (1968).
77. A. K. Bruce, P. A. Sansone, and T. J. MacVittie, Radiat. Res. 38, 95 (1969).
78. V. P. Golikov, V. P. Trofimov, and A. E. Kalmanson, Radiobiologiya, 9, 346 (1969); Chem. Abstr., 71, 88232 (1969).
79. L. S. Meyers, M. L. Hollis, and L. M. Theard, Advan. Chem. Ser., 1968, No. 81, 345; Chem. Abstr., 69, 65006 (1968).
80. S. C. Lillicrap and E. M. Fielden, J. Chem. Phys., 51, 3503 (1969).
81. J. Blok and W. S. D. Verhey, Radiat. Res., 34, 689 (1968).
82. P. Kenny and B. Commoner, Nature, 223, 1229 (1969).
83. A. Pihl, T. Henriksen, and T. Sanner, Radiat. Res., 35, 235 (1968).
84. P. Milvy and I. Pullman, ibid., 34, 265 (1968).
85. V. T. Andrianov, A. N. Pisarevskii, D. M. Spitkovskii, and V. I. Krot, Radiobiologiya, 9, 302 (1969); Chem. Abstr., 71, 27419 (1969).
86. P. E. Brown, Radiat. Res., 34, 24 (1968).
87. T. C. Owen and A. C. Wilbraham, J. Amer. Chem. Soc., 91, 3365 (1969).
88. Z. M. Bacq, M. L. Beaumariage, R. Goutier and P. Van Caneghem, Brit. J. Pharmacol., 34, 202P (1968); Chem. Abstr., 70, 641 (1969).
89. J. R. Maisin, Eff. Radiat. Cell. Proliferation Differentiation, Proc. Symp., 1968, 541; Chem. Abstr., 70, 17045 (1969).
90. A. S. Arkhipov, Y. P. Kozlov, and E. V. Burlakova, Radiobiologiya, 7, 623 (1967); Chem. Abstr., 68, 689 (1968).
91. G. A. Taranenko, Y. P. Kozlov, and N. N. Lukin, Tr. Mosk. Obschest. Ispyt. Prir., 28, 250 (1968); Chem. Abstr., 69, 83928 (1968).
92. S. E. Manoilov, Tr. Leningrad Khim.-Farm. Inst., 20, 131 (1967); Chem. Abstr., 69, 49642 (1968). See also ibid., 20, 142 (1967); Chem. Abstr., 68, 49643 (1968).
93. L. Lohmann, M. Momeni, and P. Nette, Strahlentherapie, 134, 590 (1967); Chem. Abstr., 68, 66188 (1968).
94. W. O. Foye and M. C. Solis, J. Pharm. Sci., 58, 352 (1969); Chem. Abstr., 71, 11592 (1969).
95. H. Modig, Biochem. Pharmacol., 17, 177 (1968); Chem. Abstr., 68, 84789 (1969). See Nature, 217, 1209 (1968) for a discussion.
96. H. Modig and L. Revesz, Arzneimittel-Forsch., 18, 1156 (1968), Chem. Abstr., 69, 103612 (1968).

97. H. Modig and L. Revesz, Int. J. Radiat. Biol., 13, 469 (1967); Chem. Abstr., 69, 41578 (1968).
98. A. G. Tarasenko, H. T. Le, E. V. Gusareva, and E. Y. Graevskii, Dokl. Akad. Nauk SSSR, 178, 951 (1968); Chem. Abstr., 68, 102383 (1968).
99. L. A. Tiunov and G. A. Vasil'ev, Radiobiologiya, 8, 756 (1968); Chem. Abstr., 70, 44640 (1969).
100. M. L. Freedman, P. L. Freedman, and A. K. Bruce, Nature, 224, 183 (1969).
101. W. K. Sinclair, Radiat. Res., 39, 135 (1969).
102. Z. Nagy, P. Kovacs, C. Balazs, and F. Hernandi, Biochem. Pharmacol., 17, 861 (1968); Chem. Abstr., 69, 33369 (1968).
103. J. W. Harris, R. B. Painter, and G. M. Hahn, Int. J. Radiat. Biol., 15, 289 (1969); Chem. Abstr., 71, 36289 (1969).
104. E. N. Goncharenko, Y. B. Kudryashov, and L. I. Alieva, Dokl. Akad. Nauk SSSR, 184, 1437 (1969); Chem. Abstr., 70, 112161 (1969).
105. D. D. Reynolds and D. L. Fields, Brit. Patent, 1,141,749 (1969); Chem. Abstr., 70, 99623 (1969).
106. L. R. Stromberg, M. M. McLaughlin, and R. M. Donati, Proc. Soc. Exp. Biol. Med., 129, 140 (1968); Chem. Abstr., 70, 682 (1969).
107. H. Laborit, C. Baron, B. Weber, and M. Corbel, Agressologie, 10, 189 (1969); Chem. Abstr., 71, 59173 (1969); ibid., 10, 205 (1969); Chem. Abstr., 71, 59174 (1969).
108. A. Comfort, Nature, 217, 320 (1968).
109. N. P. Buu-Hoi, Int. Congr. Gerontol., Proc., 7th, 1966, 1 and 219; Chem. Abstr., 71, 28892 (1969).
110. D. Harman, Radiat. Res., 35, 547 (1968).
111. J. R. Maisin, A. Dunjic, and G. Mattelin, Strahlenschutz Forsch. Prax., 6, 125 (1966); Chem. Abstr., 69, 64974 (1968).

Chapter 33. Reactions of Interest in Medicinal Chemistry

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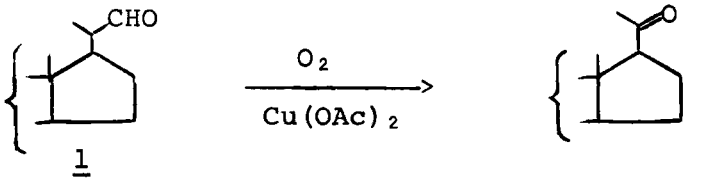
General - The year 1969 has been marked by the appearance of a new journal (Synthesis) covering synthetic organic methods and by volume 2 of the Fiesers' Reagents In Organic Synthesis.[1] The latter contains a remarkable number of references to 1969 papers, and due to space limitation an effort has been made to cover here only 1969 work which does not appear in the Fieser book.

Oxidations - The properties of 1-chlorobenzotriazole, capable of effecting high-yield oxidations of alcohols to aldehydes and ketones as well as other reactions, have been further described.[2] Oxidation occurs on refluxing in CCl_4 for a short time, and is believed to occur via a radical chain mechanism.

Branched aldehydes such as 1 are converted to ketones in 90% yield by O_2 in the presence of $Cu(OAc)_2$, DABCO, and 2,2'-bipyridyl.[3]



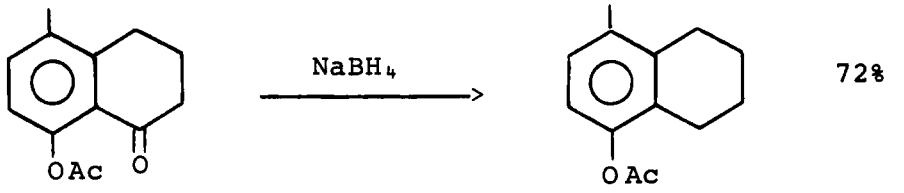
1

A large number of 1,4-, 1,5-, and 1,6-diols is converted to lactones on treatment with silver carbonate on celite in refluxing benzene: mevalonolactone is thus prepared in 74% yield.[4]

An ingenious method for oxidation of primary amines modelled after biological transamination reactions has been developed.[5] The readily available 3,5-di-5-butyl-1,2-benzoquinone is used to oxidize amines R_2CHNH_2 to ketones R_2CO in 70-90% yield, and a series of mesitylglyoxals to oxidize amines RCH_2NH_2 to aldehydes RCHO in 55-90% yield.

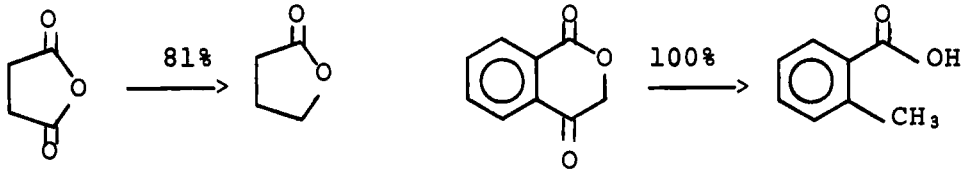
Reductions - The use of AlH_3 selectively to reduce the carbonyl group in a series of Δ^2 cyclopentenones has been described.[6] Selective reduction of the carbonyl group in α,β unsaturated aldehydes is reported, using 5% Osmium on Carbon as hydrogenation catalyst.[7]

$NaBH_4$ has been reported useful for the high-yield transformation of o- or p-hydroxy or acetoxy aryl ketones to the corresponding hydrocarbons.[8,9] If the functional groups are



are meta, the carbinol is produced. Steric effects have a profound influence on the rate of reduction of methyl esters by $NaBH(OMe)_3$.[10] The relative rate is 1° > 2° > 3°, and preparative selectivity is possible.

A special $Pd(OH)_2$ catalyst is used to reduce selectively one carbonyl group of cyclic anhydrides.[11]



Et_3SiH in the presence of Pd has been reported to be usable in a convenient way to effect the conversion of acyl chlorides to aldehydes in higher yields than are obtainable with lithium hydrides.[12]

Nitriles may be converted to secondary amines via nitrilium salts 2.[13] Depending upon the choice of oxonium salt, a variety of alkyl groups may be introduced.

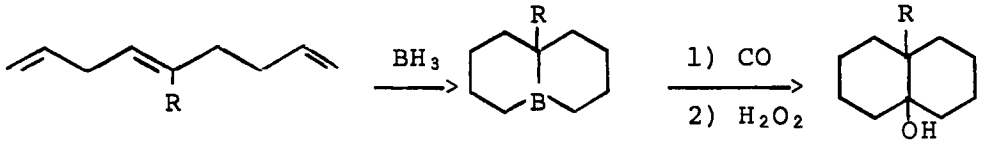
$$RCN + Et_3O^+ BF_4^- \longrightarrow \underset{BF_4^-}{RC{=}\overset{+}{N}HCH_2CH_3} \xrightarrow[2)\ NaBH_4]{1)\ EtOH} RCH_2NHC_2H_5$$

2

The isolation of $[Py_2 \cdot DMF \cdot RhCl_2(BF_4)]$, a highly active chloroform-soluble catalyst which gives the same product stereochemistry observed in heterogenous hydrogenation, has been described.[14] The stereoselective addition of H_2 or D_2 to 1,3-butadiene systems can be induced with a tricarbonyl chromium complex.[15]

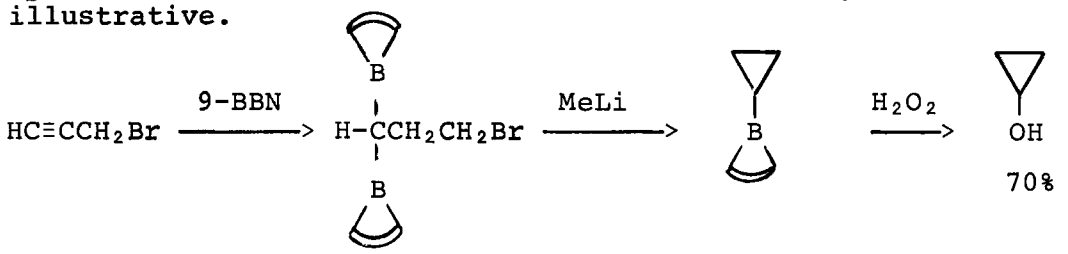
Hydrogenolysis of alkyl halides may be performed by using $NaBH_4$ in DMSO,[16] and of aryl halides by $LiAlH_4$ in THF,[17] A method for generating organotin hydrides *in situ* for use in hydrogenolysis reactions of alkyl halides has been presented.[18]

Carbocylic Ring Formation - The bicyclic borane 4 can be easily prepared from the linear triene 3 and converted into the alcohol 5 in 78% overall yield.[19]



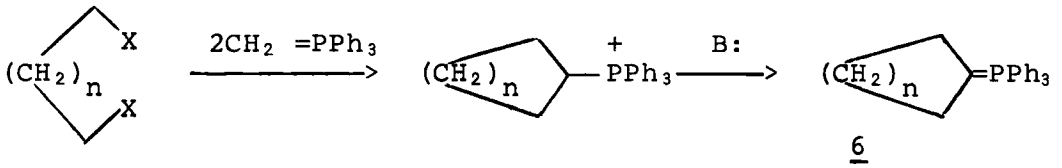
3 4 5

The uses of 9-borabicyclo[3,3,1]nonane (9-BBN) have been extended to synthesis of various cyclopropane[20,21] and cyclobutane[20] derivatives, of which the following is illustrative.



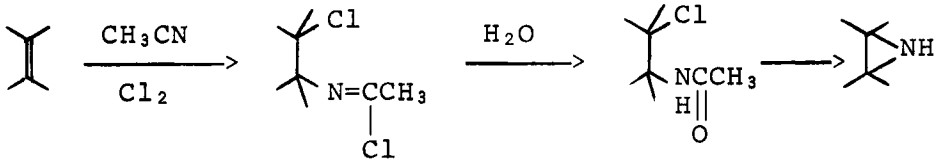
Bis-(bromomethyl)mercury converts various olefins into cyclopropanes in high yields;[22] the reactions seem less sensitive to steric effects than the Simmons-Smith procedure. Methylcyclopropanes have been obtained in far higher yields than those afforded by Simmons-Smith conditions (45-96%) from olefins and 1,1-diiodoethane in the presence of diethylzinc.[23] Similarly, carbenoid (as opposed to carbene) decomposition of aryldiazomethanes in the presence of olefins, zinc halides, and lithium halides, yields cyclopropanes in 40-90% yield.[24] In both reactions catalyzed by zinc, the predominant product from *cis*-olefins is the thermodynamically unstable *syn* cyclopropane. A photochemical method of generating phenylcarbene from phenyltetrazole anion has been described.[25] Cyclopropanes are formed in a non-stereospecific manner when this reagent is allowed to react with olefins.

Cyclobutanones have been prepared from vinylic cyclopropanols,[26] and the unique ylids 6, prepared as shown, can be converted into a wide variety of carbocyclic systems.[27]

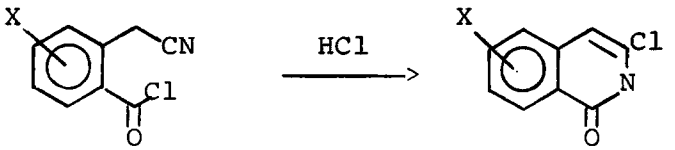


6

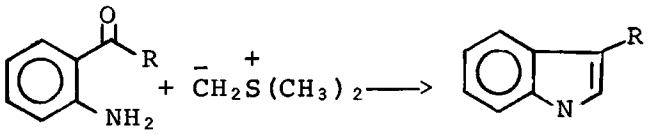
Heterocyclic Ring Formation - Aziridines can be formed in a regio- and stereoselective manner by LAH reduction of the β-iodoazides obtained by addition of INCO to olefins.[28] The reaction has been used to form 16β,17β-aziridines in steroids.[29] Treatment of olefins with acetonitrile and chlorine yields aziridines in moderate yield as shown below.[30]



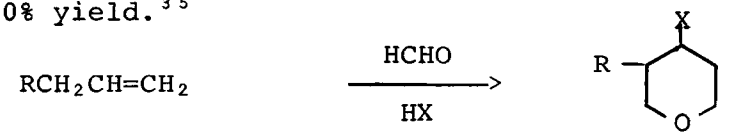
A new,more versatile isoquinoline synthesis has been reported.[31]



Dimethylsulfonium methylide reacts with o-aminoaryl ketones to yield substituted indoles.[32]

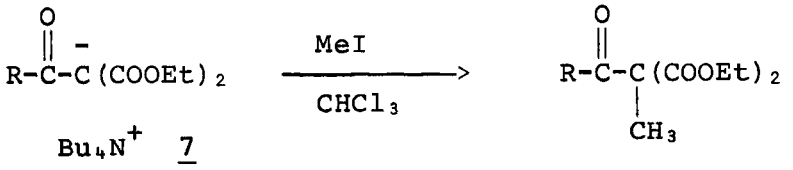


As an alternative to Corey's reagent, methylene dibromide in the presence of Li may be used to convert aldehydes and ketones to epoxides in 35-90% yield.[33] Oxetanes are formed in 80-90% yield by heating 3-bromo-1-alkoxytributyltin compounds.[34] If olefins are treated under "anhydrous" Prins conditions, tetrahydropyrans are obtained in 50-80% yield.[35]



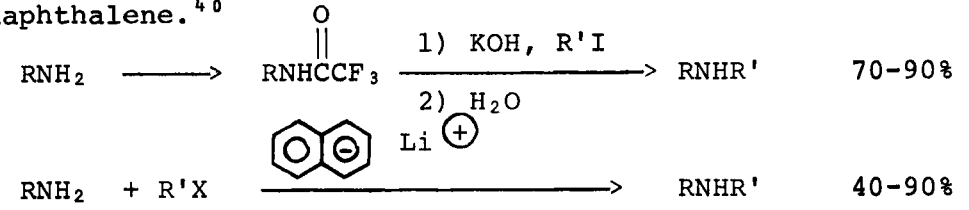
Alkylation Reactions - Anions whose nucleophilicity is too poor to allow alkylation reactions to occur under normal

conditions (alcohol, strong base), can be successfully alkylated following extraction of their tetrabutylammonium salts into chloroform solution. For example, the acylmalonic ester 7 was methylated in quantitative yield.[36] It is also convenient to alkylate methyl acetoacetate in this fashion.[37]

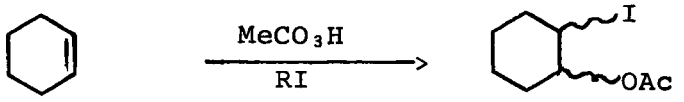


Magnesium enolate salts, prepared in hexamethylphosphoramide using Grignard reagents, afford in many cases much higher yields of C-alkylated products than can be obtained by conventional methods, and extend the range of alkylating agents which can be used.[38]

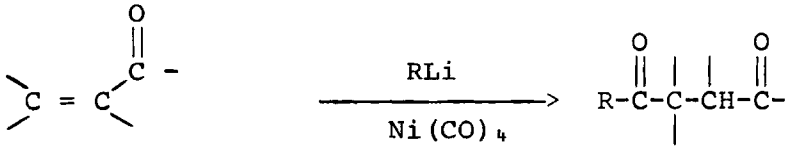
Amines may be cleanly N-alkylated via the trifluoroacetamide derivative,[39] or using as catalyst the lithium salt of naphthalene.[40]



Additions to Carbonyl and Olefinic Linkages - The Reformatsky reaction yields β-hydroxy esters readily, but these are often difficult to hydrolyze. If a Reformatsky reagent prepared from an α-bromo t-butyl ester is employed, the β-hydroxy acid is obtained directly.[41] It is also possible to prepare Grignard reagents from t-butyl bromoacetates, and these give exclusively axial addition to the carbonyl groups of a number of carbocyclic ketones.[42] Very high α,β/β,γ specificity in the preparation of olefinic esters via the Reformatsky reaction may be obtained if the intermediate β-hydroxy esters are converted to acetates and pyrolyzed.[43] Nitroacetic acid will add the elements of nitromethane across aldehyde carbonyl groups upon heating, thus allowing this reaction to be performed without strong base.[44] Peracetic acid-alkyl iodide mixtures will add the elements of acetyl hypoiodite across simple olefins.[45] Organolithium reagents do not add



to isolated olefinic linkages, but additions to allylic alcohols may be performed.[46] The intermediate lithium salts may be hydrolyzed to yield 2-alkylpropanols, or carbonated to afford γ-lactones. Some additions are greatly facilitated by tetramethylethylenediamine, as are many other reactions of organolithium reagents. The stereochemistry of addition of lithium reagents to allylic alcohols has been found to be the opposite of that observed with Grignard reagents.[47] 1,4-Dicarbonyl systems may be obtained by interaction of α,β unsaturated ketones, organolithium reagents, and $Ni(CO_4)$.[48]



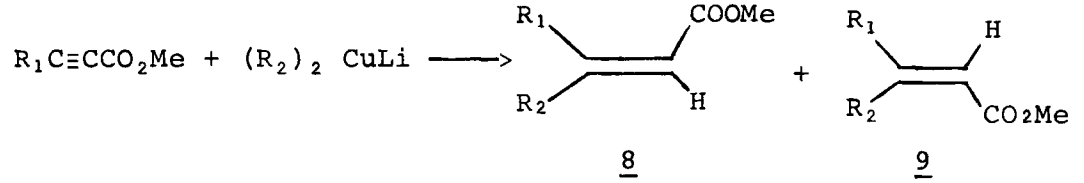
The latter reagent also catalyzes the carbonylation of vinylic bromides.[49] A similar carbonylation may be performed on aromatic olefins using a $PdCl_2 \cdot Et_3N$ complex as catalyst.[50]

Trialkylboranes or alkyl 9-borabicyclo[3,3,1]nonanes, obtained by appropriate olefin addition reactions, may be used in S_N2 displacement reactions upon α-halo esters,[51,52] α-haloketones,[53] or α-halonitriles[54] to effect alkylation of these substrates in high yield. In many cases 2,6-di-t-butylphenoxide, a base of very high steric requirement, is essential.

Hydroformylation of olefins may be conducted at low pressure, using $(PhO)_3P$ as catalyst.[55]

Organometallic Reagents - Thallium (I) carboxylates, which are easily prepared and purified and are stable indefinitely, undergo the Hunsdiecker reaction in approximately three times the yield afforded by silver salts.[56] Thallium salts are also useful in alkylation of purines,[57] and in synthesis of aryl iodides[58] and bromides[59] as well as aliphatic bromides.[60]

An interesting stereospecific synthesis of olefinic esters has been reported.[61,62] At -78°, the cis adduct 8 is obtained in 99% yield, whereas at higher temperature a mixture is obtained, of which the trans adduct 9 comprises 90%. A similar series of reactions performed on acetylenic acids has also been described.[63] A review of reactions



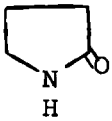
8 9

possible with lithium diaryl- and dialkylcuprates has been presented.[64]

Allyl Grignard reagents may be used as nucleophiles with good results in the presence of hexamethylphosphoramide.[65]

Miscellany - The use of borate esters to protect alcohols during synthetic manipulations is a more versatile technique than expected. Borates are stable to anhydrous acidic or basic conditions and are rapidly hydrolyzed in water.[66]

Eneamines of lower aliphatic aldehydes can be prepared in quantitative yield using tris(dimethylamino)methane.[67]

Pyrollidone hydrotribromide [ $\cdot Br_2HBr$] is a

remarkably selective brominating agent, for which the order of substrate reactivity is ketones >> olefins >> enol acetates.[68] Preparative selectivity is possible.

Triphenylphosphine dibromide is a versatile dehydrating agent for amides. Depending on the starting material, nitriles, carbodiimides, isonitriles, or keteneimines may be obtained.[69] Silicon tetrachloride is a useful coupling agent for amide formation, since it is converted to silica, easily separable from other reaction products.[70]

A number of extensions and modifications of the Vilsmeier reaction are reported.[69,71,72,73]

Selective N-dealkylation in the presence of N-aryl linkages may be induced by pyridine hydrochloride,[74] and selective N-debenzylation by thiophenoxide anion.[75]

References

1. M.Fieser and L.F.Fieser,"Reagents for Organic Synthesis," Volume 2, New York, Wiley, 1969.
2. C.W.Rees and R.C.Storr, J.Chem.Soc., C, 1474 (1969).
3. V.van Rheenan, Tetrahedron Lett., 985 (1969)
4. M.Fetizon,M.Goldfier, and J-M.Louis, J.Chem.Soc.,D,1118 (1969).
5. E.J.Corey, K.Achiwa, J.Amer.Chem.Soc., 91, 1429 (1969).
6. H.C.Brown and H.M. Hess, J.Org.Chem., 34, 2206 (1969).
7. P.N.Rylander and D.P.Steele, Tetrahedron Lett.,1579 (1969).
8. B.J. McLoughlin, J.Chem.Soc.,D,541 (1969).
9. K.H.Bell, Aust. J. Chem., 22, 601 (1969).
10. R.A.Bell and M.B.Gravestock, Can.J.Chem.,47, 2099 (1969).
11. R.Kuhn and I.Butula, Justus Liebigs Ann.Chem.,718,50(1968).

12. J. D. Citron, J. Org. Chem., 34, 638 (1969).
13. R. F. Borch, ibid., 34, 627 (1969).
14. I. Jardine and F. J. McQuillen, J. Chem. Soc., D, 477 (1969).
15. E. N. Frankel and R. O. Butterfield, J. Org. Chem., 34, 3930 (1969).
16. H. M. Bell, C. W. Vanderslice and A. Spehar, ibid., 34, 3923 (1969).
17. H. C. Brown and S. Krishnamurthy, ibid., 34, 3918 (1969).
18. G. L. Grady and H. G. Kuvila, ibid., 34, 2014 (1969).
19. H. C. Brown and E. Negishi, J. Amer. Chem. Soc., 91, 1224 (1969).
20. H. C. Brown and S. P. Rhodes, ibid., 91, 4306 (1969).
21. H. C. Brown and S. P. Rhodes, ibid., 91, 2149 (1969).
22. D. Seyfurth, R. M. Turkel, M. A. Eisert, and L. J. Todd, ibid., 91, 5027 (1969).
23. J. Nishimura, N. Kawabata, and J. Furakawa, Tetrahedron, 25, 2647 (1969).
24. S. H. Goh, L. E. Closs and G. L. Closs, J. Org. Chem., 34, 25 (1969).
25. P. Schneider, ibid., 34, 199 (1969).
26. R. E. Cochoy, and M. S. Baird, J. Amer. Chem. Soc., 91, 2375 (1969).
27. H. J. Bestmann and E. Krantz, Chem. Ber., 102, 1802 (1969).
28. A. Hassner, G. J. Matthews and F. J. Fowler, J. Amer. Chem. Soc., 91, 5046 (1969).
29. G. J. Matthews and A. Hassner, Tetrahedron Lett., 1833 (1969).
30. G. Lamaty, A. Delbord, and W. Werner, Justus Liebigs Ann. Chem., 726, 77 (1969).
31. G. Simcheu and W. Kramer, Chem. Ber., 102, 3656 (1969).
32. P. Bravo, G. Gandiano, A. Umani-Ronchi, Tetrahedron Lett., 679 (1969).
33. F. Bertini, P. Graselli, and G. Zubani, J. Chem. Soc., D, 1047 (1969).
34. B. Delmond, J. C. Pommier, and J. Valade, Tetrahedron Lett., 2089 (1969).
35. P. A. Stapp, J. Org. Chem., 34, 479 (1969).
36. A. Brandstrom and U. Junggen, Acta Chem. Scand., 23, 2536 (1969).
37. A. Brandstron and U. Junggen, ibid., 23, 2204 (1969).
38. J. Fauvarque and J-F. Fauvarque, Bull. Soc. Chim. Fr., 160 (1969).
39. R. A. W. Johnstone, D. W. Payling and C. Thomas, J. Chem. Soc., C, 2223 (1969).
40. K. Suga, S. Watanabe, T. P. Pau and T. Fujita, Chem. and Ind., 78 (1969).
41. A. E. Opara and G. Read, J. Chem. Soc., D, 679 (1969).
42. M. Perry and Y. Marconi-Barand, Bull. Soc. Chim. Fr., 2372 (1969).

43. K. H. Fung, K. J. Schmalzl, and R. N. Mirrington, Tetrahedron Lett., 5017 (1969).
44. W. L. F. Armarego, J. Chem. Soc., C, 986 (1969).
45. Y. Ogata and K. Aoki, J. Org. Chem., 34, 3978 (1969).
46. J. K. Crandall and A. C. Clark, Tetrahedron Lett., 325 (1969).
47. H. Felkin, G. Swierczewski and A. Tambute, ibid., 707 (1969).
48. E. J. Corey and L. S. Hegedus, J. Amer. Chem. Soc., 91, 4926 (1969).
49. E. J. Corey and L. S. Hegedus, ibid., 91, 1233 (1969).
50. T. Yukawa and S. Tsutsumi, J. Org. Chem., 34, 738 (1969).
51. H. C. Brown, H. Nambu, and M. M. Rogic, J. Amer. Chem. Soc., 91, 6856 (1969).
52. H. C. Brown and M. M. Rogic, ibid., 91, 2146 (1969).
53. H. C. Brown, H. Nambu and M. M. Rogic, ibid., 91 6852 (1969).
54. H. C. Brown, H. Nambu and M. M. Rogic, ibid., 91, 6854 (1969).
55. R. L. Pruett and J. A. Smith, J. Org. Chem., 34, 327 (1969).
56. A. McKillop, D. Bromley and E. C. Taylor, ibid., 34, 1172 (1969).
57. E. C. Taylor, Y. Maki and A. McKillop, ibid., 34, 1170 (1969).
58. A. McKillop, J. S. Fowler, M. J. Zelesko, J. D. Hunt, E. C. Taylor and G. McGillivray, Tetrahedron Lett., 2427 (1969).
59. A. McKillop, D. Bromley and E. C. Taylor, ibid., 1623 (1969).
60. A. McKillop, D. Bromley and E. C. Taylor, J. Org. Chem., 34, 1172 (1969).
61. E. J. Corey and J. A. Katzellenbogen, J. Amer. Chem. Soc., 91, 1851 (1969).
62. J. B. Siddall, M. Biskup, and J. H. Fried, ibid., 91 1853 (1969).
63. J. Klein and R. M. Turkel, ibid., 91, 6186 (1969).
64. G. M. Whitesides, W. F. Fisher, J. S. Fillippo, Jr., R. W. Baske and H. O. House, ibid., 91, 4871 (1969).
65. G. Stork, P. A. Greico, and M. Gregson, Tetrahedron Lett., 1393 (1969).
66. W. I. Fanta and W. F. Erman, ibid., 4155 (1969).
67. H. G. Hanthal and D. Schied, Z. Chem., 9, 62 (1969).
68. D. V. C. Awang and S. Wolfe, Can. J. Chem., 47, 706 (1969).
69. H. J. Bestmann, J. Lienert, and L. Mott, Justus Liebigs Ann. Chem., 718, 24 (1968).
70. T. H. Chan and L. T. L. Wong, J. Org. Chem., 34, 2766 (1969).
71. K. Ikawa, F. Takami, Y. Fukui and K. Takuyama, Tetrahedron Lett., 3279 (1969).

72. J. G. Dingwall, D. H. Reid and K. Wade, J. Chem. Soc., C, 913 (1969).
73. D. E. Horning and J. M. Muchowski, Can. J. Chem., 48, 193 (1970).
74. N. P. Buu-Hoi, G. Saint-Ruf and B. Lobert, Bull. Soc. Chim. Fr., 1769 (1969).
75. T. Kametani, K. Kigasawa, M. Hiragi, N. Wagatsuma and K. Wakisaka, Tetrahedron Lett., 635 (1969).

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