CONTRIBUTORS

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PREFACE

Readers will find again this year a deliberate attempt to introduce new and timely topics on a one-time or intermittent basis. In Volume 8, fifteen of the thirty-four chapter topics were not discussed in the previous volume.

The number of medical fields in which it is becoming possible to discuss mechanisms at the molecular or enzymatic level is increasing at an accelerating rate. In the present volume, at least nine chapters (3, 9, 10, 11, 18, 23, 27, 28, 29) include a significant discussion of cyclic AMP, at least four (9, 11, 18, 22) discuss involvement of endogenous prostaglandins and at least three (16, 17, 29) discuss the role of interferon in resistance to infection. The story of apparent involvement of viruses in the development of neoplasms via molecular biological mechanisms continues to unfold and is discussed in several chapters.

The interrelationship of diseases through common or similar mechanisms causes redundancy problems for chapter authors and editors. On the other hand, this redundancy serves to emphasize the commonality of many so-called distinct diseases and teaches the medicinal chemist that he must follow the literature in related fields as well as his own. To this end Annual Reports may be able to serve an increasingly useful purpose.

Kalamazoo, Michigan
June, 1973

Richard V. Heinzelman

Section I - CNS Agents

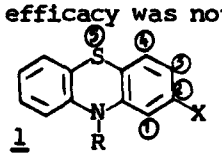
Editor: Edward L. Engelhardt
Merck Sharp and Dohme Research Laboratories, West Point, Pa. 19486

Chapter 1. Antipsychotic and Antianxiety Agents

Charles L. Zirkle and Carl Kaiser
Smith Kline & French Laboratories, Philadelphia, Pa. 19101

Introduction - Two drugs belonging to these classes were marketed in the United States in 1972; these are fluphenazine decanoate, a new ester of fluphenazine with utility in antipsychotic maintenance therapy, and clorazepate dipotassium,[1] an antianxiety drug of the benzodiazepine type. Structure-activity relationships (SAR) among antipsychotic and psychotropic agents,[2-5] their mechanism of action,[6-8] clinical utility,[9,10] pharmacological actions[11] and methods for their pharmacological evaluation[12] were subjects of recent reviews. Most research on the actions of antipsychotics still centers on the dopaminergic (DA) blocking effects of these drugs.[13] A most interesting report describes a DA-sensitive adenylate cyclase in the neostriatum that is suggested to be a DA receptor.[14]

Tricyclic antipsychotics - The use of long-acting fluphenazine[15] in the management of schizophrenia and its comparison with conventional therapy was reviewed.[16] Studies of other 6-6-6 tricyclics have included several new phenothiazine derivatives. A diazabicyclononane analog 1a of chlorpromazine (CPZ), one of a series, presented a typical neuroleptic profile.[17] The sulfone derived from thioridazine, i.e., inofal (1b), caused improvement in two-thirds of a group of chronic schizophrenic females.[18] A valeroylphenothiazine, M&B 18,706 (1c), was a selective and more potent fusimotor depressant than CPZ.[19] In SAR studies of phenothiazines 1, [X=H, 2- or 3-Cl, 2-CF_3, 2-Cl-5→O; R=$(CH_2)_3N(CH_3)_2$] a high oil: water partition coefficient (which has been associated with neuroleptic potency) was related to toxicity in a goldfish test[20] and to serum albumin binding.[21] Numerous tricyclic compounds were examined for their ability to inhibit glutamate dehydrogenase; however, a clearcut correlation with antipsychotic efficacy was not observed.[22]



1

a) X=Cl; R=$(CH_2)_3$N⟨⟩NCH$_3$

b) X=SO_2CH_3; R=$(CH_2)_2$⟨N⟩CH$_3$

c) X=$CO(CH_2)_3CH_3$; R=$CH_2CH(CH_3)CH_2N(CH_3)_2$

d) X=Cl; R=$(CH_2)_3NH(CH_2)_2NH_2$

e) X=CF_3; R=$(CH_2)_3NH(CH_2)_2NH_2$

f) X=OCH_3; R=$CH_2CH(CH_3)CH_2NHCH_3$

g) X=OCH_3; 5→O; R=$CH_2CH(CH_3)CH_2N(CH_3)_2$

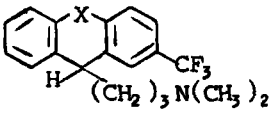
h) X=OH, 5→O; R=$CH_2CH(CH_3)CH_2N(CH_3)_2$

i) X=OCH_3, 5→O; R=$CH_2CH(CH_3)CH_2NHCH_3$

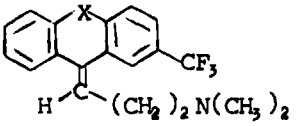
Metabolic studies of piperazine-bearing phenothiazine antipsychotics in rats and dogs demonstrated the formation of ethylenediamine derivatives 1d and 1e from prochlorperazine and trifluoperazine, respectively.[23] Levo-

mepromazine metabolites isolated from human urine were RP 16584 (1f), RP 19609 (1g), RP 21682 (1h)[24] and the demethyl sulfoxide 1i.[25] Some new types of CPZ metabolites were isolated from red blood cells. The drug was found here as a mixture of N-hydroxynorchlorpromazine and its sulfoxide. Additional amounts of these metabolites were present as conjugated forms.[26]

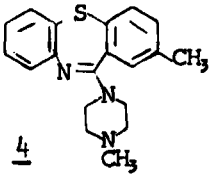
Other 6-6-6 compounds included a series of aminoalkyl- and aminoalkylidene-substituted xanthenes, thioxanthenes, and related compounds. The aminopropyl derivatives 2a and 2b were nearly equipotent with CPZ in several neuropharmacological tests, whereas the cis (Z)-aminopropylidene analogs 3a and 3b were even more potent.[27]



2 a) X=O. b) X=S



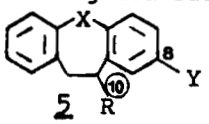
3 a) X=O. b) X=S



4

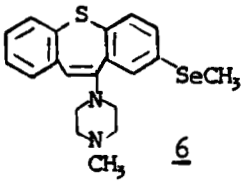
Among antipsychotic 6-7-6 compounds considerable interest has focused on clozapine which does not cause catalepsy or antagonize apomorphine-induced emesis.[13] It increased cerebral turnover of DA in rats, antagonized prochlorperazine-induced catalepsy[28] and was reported to be a clinically-effective antipsychotic.[29,30] A benzothiazepine, metiapine (4) compared favorably with trifluoperazine in a double blind evaluation in chronic schizophrenics.[31] Continuing SAR studies in the 10-(4-methylpiperazinyl)-10,11-dihydrodibenzo[b,f]thiepin (perathiapine) series were described in 1972. The neuropharmacological properties of the racemic 8-Cl derivative, octoclothepine, and its enantiomers were similar.[32] Methiothepin (5a), a CH_3S congener of perathiepine, had neuroleptic activity.[33] Oxyprothepin enanthate (5b), the most extensively studied member of a series of esters of alcoholic perathiepine derivatives,[34] had a duration of neuroleptic potency comparable to that of fluphenazine enanthate in dogs, rats and rabbits.[35] The decanoate 5c (VUFB 9977) also had long-lasting neuroleptic activity in dogs and rats.[36] Introduction of an 8-acetamido (5d) substituent markedly reduced perathiepine's neuroleptic potency in rotating rod and motor activity (MA) tests in mice and in a catalepsy test in rats; however, similar CH_3Se (5e) substitution significantly increased potency. Although it was only about half as potent as the CH_3S counterpart 5a in the rotating rod test, 5e was 4 times more potent in the catalepsy assay. The unsaturated congener 6 was also very potent (4-32X CPZ) in the various neuropharmacological tests.[37] An 8-$(CH_3)_2NSO_2$ derivative, sulfamothepin (5f), had potency equal to, or greater than, that of perphenazine in these tests for neuroleptic activity in mice and rats.[38] In the same pharmacological systems several perathiepine congeners, e.g. 5g-5l, in which the 10-piperazinyl substituent is replaced by a 3,8-diazabicyclo[3.2.1]octyl system, were considerably less potent than their Cl-(octoclothepine) and CH_3S-(5a) counterparts. The congeners 5g and 5h were about half as potent as their piperazinyl parents whereas their isomers 5i and 5j were even less effective.[39] Neuroleptic, antiemetic and antiserotonin activity were claimed for the piperidinyl-substituted dibenzocycloheptane 5k;[40] however, some 'ring-opened'

congeners, e.g. 7, caused only weak depression of MA and potentiation of thiopental-induced sleep in mice.[41] Another tricyclic relative, AY-22,214 (8)[42] caused a taming effect in aggressive rats at doses well below those causing ataxia.[43]



5

a) X=S; Y=CH_3S; R=N NCH$_3$
b) X=S; Y=CH_3S; R=N N(CH$_2$)$_3$OCO-n-C$_6$H$_{13}$
c) X=S; Y=CH_3S; R=N N(CH$_2$)$_3$OCO-n-C$_9$H$_{19}$
d) X=S; Y=NHCOCH$_3$; R=N NCH$_3$
e) X=S; Y=CH_3Se; R=N NCH$_3$
f) X=S; Y=(CH$_3$)$_2$NSO$_2$; R=N NCH$_3$
g) X=S; Y=Cl; R=N NCH$_3$
h) X=S; Y=CH_3S; R=N NCH$_3$
i) X=S; Y=Cl; R=N NCH$_3$
j) X=S; Y=CH_3S; R=N NCH$_3$
k) X=CH_3; Y=Cl; R= NCH$_3$



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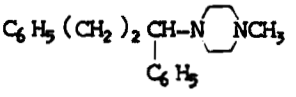

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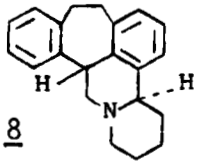

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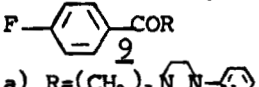
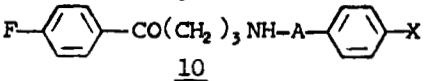
Butyrophenones, β-aminoketones, aralkylamines and related compounds - SAR, syntheses, receptor binding, metabolism and clinical utility of the butyrophenone-type antipsychotic agents have been reviewed.[44,45] A summary of 15 years clinical experience with haloperidol was presented.[46] The distribution and metabolism of azaperone (9a), a neuroleptic which induces rapid sedation,[47] was studied in rats and pigs.[48] In a series of cyclopropyl analogs of antipsychotic butyrophenones greatest potency was demonstrated by 9b, which was equipotent with trifluperidol (10X CPZ) in a test for depression of MA in mice.[49] Some secondary 4-aminobutyrophenone derivatives, 10a and 10b, were considerably more potent than CPZ in behavioral (loss of righting reflex, traction, chimney, pedestal and nicotine antagonist) tests in mice.[50] In a related saturated (cyclohexyl) series, the trans derivative 10c was one of the most potent compounds, based on overt end points and in blocking uptake of NE and 5-HT by the heart and spleen. It was more potent than the cis-isomer (>10X), 10a, and 10b in most tests.[50,51] The most effective members of a series of butyrophenone-related 8-azaspiro[4.5]decane-7,9-diones were 11a-11c, which induced CAR blockade (>CPZ) in rats and caused tranquilization in monkeys.[52] Of particular interest was 11b (MJ 9022-1) which, despite its potent neuroleptic activity, caused only mild sedation and hypothermia and was almost devoid of analgetic and α-adrenergic actions.[53]



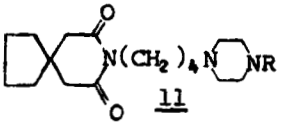
9

a) R=(CH$_2$)$_3$N N-
b) R= CH$_2$N OH C$_6$H$_4$-4-F



10

a) A= ; X=F
b) A= ; X=SCH$_3$
c) A= ; X=F

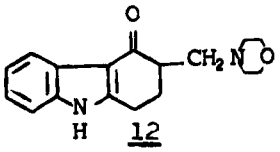


11

a) R=2-pyridyl
b) R=2-pyrimidyl
c) R=4-CH$_3$-2-pyrimidyl

In a series of 2,3-dihydro-4(1H)carbazolones the most potent member was 12, a structural relative of molindone. It decreased MA in mice

(MED=4 mg/kg, i.p.).[54] Several members of another series of β-aminoketones, the piperazines 13, caused neuroleptic effects (ca. 0.1X CPZ) in several mouse tests.[55] Anti-aggressive activity in mice was produced by YG 19'256 (14)[56] which was clinically effective in aggressive oligophrenics.[57] Another β-aminoketone 15a showed a high degree of activity (0.3-0.5X CPZ) in tests for decreased MA, inhibition of mouse fighting behavior and in a CAR test in rats.[58] In general the alcoholic derivatives 15b and 15c were more potent depressants of MA in mice than their ketonic precursors. Thus 15b was equipotent with CPZ in this test, although its overall pharmacological profile was more akin to that of chlordiazepoxide. The alcohol 15c was equipotent with CPZ in a CAR test in rats.[58] Another piperazine derivative, mepiprazol (16), produced improvement in 64% of chronic schizophrenics, but placebo was effective in 48% of the group.[59]



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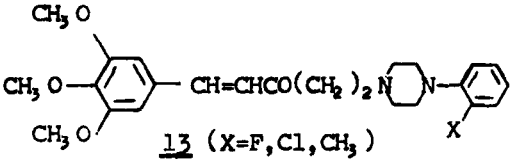
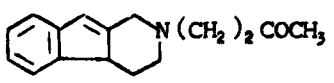


13 (X=F,Cl,CH_3)



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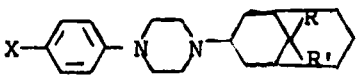
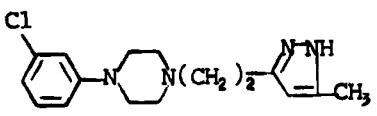


15

a) X=F; R,R'=O
b) X=H; R=OH; R'=C_6H_5
c) X=H; R=OH; R'=H



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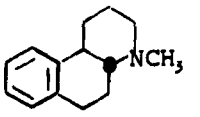
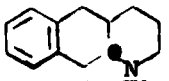


17

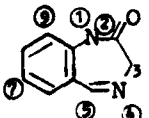


18

To determine if the cisoid or transoid phenethylamine moieties are responsible for the catalepsy and EEG changes induced by bulbocapnine[60] in animals, transoid tricyclic phenethylamines 17 and 18, as well as two cisoid relatives were studied. Potent cataleptic activity was noted for the transoid compounds, but not for their cisoid relatives. Also the transoid derivatives were much more effective in blocking the response of rat vas deferens to DA.[61] (-)-Nuciferine, (-)-5,6-dimethoxyaporphine, the most potent of a series of six, presented a neuroleptic- and, to a lesser extent, a morphine-like pharmacological profile. Although considerably less potent than CPZ, it blocked a CAR, prevented the convulsant effects of tryptamine, and protected against amphetamine-induced lethality in rats.[62]

Benzodiazepines and related compounds - Recent reviews have treated the pharmacological, biochemical, metabolic,[63] clinical aspects[64] and SAR[65,66] of benzodiazepines with antianxiety activity. Clorazepate dipotassium,[1] marketed in the United States in 1972, was studied additionally in the clinic, e.g. [67-71] in animals[72] and was reviewed.[73] Extensive pharmacological examination of a 5-(2,6-difluorophenyl) derivative 19a indicated it was more potent than diazepam [6X diazepam in a pentylenetetrazol (PTZ)-antagonism test in mice].[74] S-1530 (19b) was effective as a depressant of

spontaneous MA, in inclined plane, fighting and rotarod tests in mice (5-10X diazepam). It was also effective in an anti-PTZ assay (2X diazepam), but was less active in a CAR test in rats (0.3X diazepam).[75] Substitution of a cyano group (19c, Ro 5-4528) for diazepam's 7-Cl resulted in retention or enhancement of potency in various behavioral tests; however, 19c was only half as potent as the parent in a mouse anti-PTZ test.[76] In a series of 1-carbamoylbenzodiazepines, 19d was the most potent compound. It was equipotent with, or more potent than, diazepam in a battery of tests for antianxiety activity, but it was not as effective as an antagonist of PTZ-induced clonic convulsions in mice.[76] Potent CNS depressant activity (ca. 0.3X diazepam) was also shown by 19e in tests measuring anticonvulsant end points.[76] An N-cyclopropyl relative, ciprazepam (19f) had antianxiety properties similar to those of chlordiazepoxide; however, a 5-cyclohexenyl derivative, nortetrazepam (19g), was more sedating than its phenyl analog.[65] The D-isomer of oxazepam hemisuccinate (19h) was much more potent than the L-form in anticonvulsive, narcosis-potentiating, and muscle relaxant tests; however, the L-isomer was more effective in depressing spontaneous MA.[77]



19

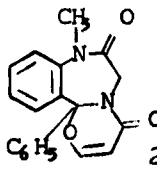
a) 1-H, 5-(2,6-$F_2C_6H_3$), 7-Cl
b) 1-CH_3, 5-C_6H_5, 7-NO_2
c) 1-CH_3, 5-C_6H_5, 7-CN
d) 1-$CONHCH_2CH{=}CH_2$, 5-C_6H_5, 7-Cl
e) 1-$CONHCH_3$, 5-C_6H_5, 7-Cl
f) 1-H, 2=NCH_2-cyclopropyl, 4→O, 5-C_6H_5
g) 1-H, 2-(1-cyclohexenyl), 7-Cl
h) 1-H, 3-$OCO(CH_2)_2CO_2H$, 5-C_6H_5, 7-Cl
i) 1-H, 4→O, 5-C_6H_5, 7-Cl, 9-OH
j) 1-H, 9-OH, 5-C_6H_5, 7-Cl
k) 1-H, 3-OH, 5-(4-HOC_6H_4), 7-Cl
l) 1-H, 3-OH, 5-(3-or 4-HO,$CH_3OC_6H_3$), 7-Cl
m) 1-H, 5-(2-pyridyl), 7-Br

In metabolism studies, two metabolites, 19i and 19j, of demoxepam were characterized by synthesis.[78] Biotransformations of oxazepam in man, pigs and rats gave 6-chloro-4-phenyl-2(1H)quinazolinone, three ring-opened metabolites, two 5-phenyl hydroxylated products, 19k and 19l, and various conjugates.[79] Urinary metabolites of bromazepam (19m) in several species included two major ring-opened products, 2-amino-5-bromobenzoylpyridine, and a glucuronide of the corresponding 3-hydroxyl derivative.[80]

Other new 1,4-benzodiazepines included ketazolam (20), said to be an effective antianxiety agent,[81] and CS-370 (21), whose pharmacological profile suggested somewhat greater potency than diazepam.[82] Synthesis of a related series of 1,2,4-oxadiazolo(4,5-d)-1,4-diazepines (22) was accomplished by 1,3-dipolar cycloaddition of appropriate nitrile oxides to 1,4-benzodiazepines.[83] In a study of a series[84] of thienodiazepinones in various tests for antianxiety potential, one of the most effective was Y-6047 (23; 2-3X diazepam in an anti-PTZ test in mice).[85] Another thienodiazepinone, QM-6008 (24) also was active in this test (0.15X chlordiazepoxide),[84] but it increased MA to a greater extent.[86]

Many N-aryl-1,5-benzodiazepinones, e.g. 25a,[87] and related N-aryl (and heteroaryl)-2,4-diones, e.g. 25b,[88] have significant antianxiety-like

pharmacological actions. Conversely, derivatives of 3,5-dihydro-4H-benzo-[2,3]diazepin-4-one, e.g. 26, differ from the 1,4-benzodiazepine anti-anxiety drugs. At doses above 250 mg/kg, p.o., 26 caused sedation and ptosis in mice.[89] SQ 20,009 (27) was studied in an attempt to correlate antianxiety and phosphodiesterase-inhibitory activities. In a punishment conflict procedure in monkeys, cats and rats it was more potent than chlordiazepoxide.[90,91]



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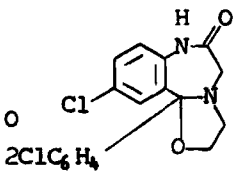

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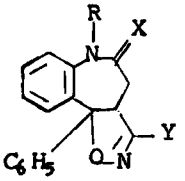

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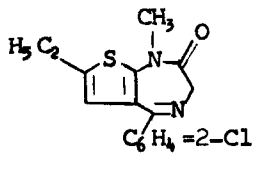
(R=H, CH_3; X=O, H_2; Y=C_6H_5, C_6H_5CO, $C(CH_3)_3$, CH_2OH)



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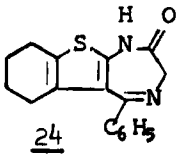

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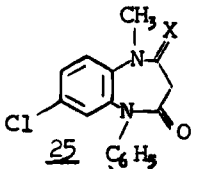
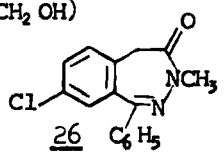


25

a) X=H_2. b) X=O



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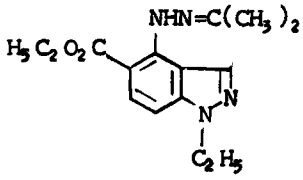

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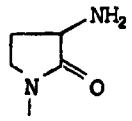

29

Other structures with antianxiety or antipsychotic activity - Several miscellaneous structures have antianxiety-like activity. A dibenzothiepine GP 41299 (28) was an effective antianxiety agent, with mild side effects, in neurotic patients receiving an average dose of 200 mg per day.[92] The pyrrolidone HA-966 (29) caused flaccid catalepsy and tranquilization at 6 mg/kg, i.v., in mice. It also decreased MA, but unlike conventional neuroleptics, had little effect on the toxicity of amphetamine in aggregated mice.[93] 5-Hydroxytryptophan, the precursor of central 5-HT, administered together with a peripheral decarboxylase inhibitor, produced improvement in 6 of 7 schizophrenic patients.[94] This combination also suppressed muricidal behavior in rats.[95] Triiodothyronine was effective in schizophrenic children who generally became less withdrawn and more responsive.[96]

β-Adrenergic blocking agents, such as propranolol which was effective in 8 of 12 schizophrenics[97] and in patients with various other psychiatric disorders,[98] have useful antianxiety properties. The central action of these drugs has been reviewed.[99] It appears that they produce their antianxiety actions via a peripheral mechanism. Thus practolol, which does not enter the CNS in significant amounts, produces notable

improvement in patients with anxiety.[100] Depression of spontaneous MA has been noted in mice treated with alprenolol, INPEA, DCI and propranolol, but not with pronethalol.[101]

Biological observations and hypotheses - Reviews[102-107] and reports continue to emphasize the "dopamine" hypothesis for schizophrenia and the anti-DA actions of the antipsychotic drugs. An abnormal dopaminergic activity may be part of the inherited vulnerability to schizophrenia.[102] This postulate links many observations from the clinical level down to the neuronal level and, perhaps, even to the molecular level if the suggestion that an adenylate cyclase may be the DA receptor in the neostriatum proves to be true.[14] Many schizophrenic patients appear to be in a state of overarousal and neuroanatomical studies indicate that CA pathways[13] are important components of arousal systems. The nigrostriatal DA pathway is viewed as a behavioral arousal and locomotor system without which practically no forms of behavior can occur. It has been suggested that the dorsal noradrenergic (NA) pathway is a part of the reticular activating system mediating tonic arousal of the cortex and that the ventral NA pathway constitutes a second arousal system involved in reinforcement or reward mechanisms.[108] However, to the considerable evidence that brain self-stimulation, which is readily antagonized by the neuroleptics, is mediated by a NA system has been added recent observations that DA pathways to the limbic forebrain and neostriatum may be involved in this behavior.[109,110] The neostriatum seems to be an important site of action of the antipsychotics, at least for the production of extrapyramidal symptoms,[13] and Klawans et al.[106] have marshalled arguments that the DA system in this center may be involved in the pathophysiology of the behavioral manifestations of schizophrenia. Although the functional roles of the mesolimbic DA system are not known, the regions of the limbic system innervated by this pathway would also seem to be likely targets for the actions of the antipsychotics.[107] It was observed that haloperidol increased turnover of DA (as indicated by increased levels of homovanillic acid) in both the limbic system and neostriatum of rabbits, but anticholinergic treatment attenuated this effect only in the striatum.[111] This finding, taken with clinical experience that antipsychotic-induced EPS can usually be alleviated without compromising the therapeutic effects, suggests that parts of the limbic system may be sites of drug action for antipsychotic activity. Amphetamine, thought to be an indirect acting DA (and NA) agent, increases arousal levels, induces stereotyped behavior, and causes psychosis resembling paranoid schizophrenia; its effects are selectively antagonized by the antipsychotics. The possible mechanisms of action of amphetamine and the implications of amphetamine psychosis as a model of schizophrenia have been extensively reviewed.[103,104] Additional studies suggest that neuronal systems other than the usually cited nigrostriatal DA pathway may be involved in the production of drug-induced stereotyped behavior and in its antagonism by neuroleptics.[112,113] The interrelationship of DA and cholinergic systems has also been studied further[112,114] and a "cholinergic-adrenergic" hypothesis of psychiatric disorders has been advanced.[115]

In recent years clinicians have been concerned with tardive dyskinesias which sometimes develop in patients on long-term neuroleptic therapy.

Paradoxically, this syndrome, prominent features of which are lingual-facial-buccal dyskinesias, produced by drugs thought to block dopaminergic activity resembles the dyskinesias produced by the dopaminergic agent L-dopa. Both types of dyskinesia are reduced by agents which deplete DA or counteract DA activity. It is suggested that the antipsychotics cause a functional denervation of DA receptors and thus receptor hypersensitivity develops leading to DA overactivity.[105,116] Amphetamine-induced stereotyped behavior, which bears some resemblance to the tardive dyskinesias, has been proposed as a model for the clinical disorder.[105]

Concerning the actions of antipsychotics at the neuronal and molecular levels, the most interesting development was a report on an adenylate cyclase identified in homogenates of the caudate nucleus of rat brain that is activated selectively by low concentrations of DA or apomorphine.[14] The stimulatory effect of DA was blocked by low concentrations of CPZ or haloperidol. Further research stemming from this provocative finding will be followed with great interest. In a thorough review Seeman[117] again points out the similarities between neuroleptics and local anesthetics suggesting that they have a common mode of action at neuronal membranes. However, York found that iontophoretically applied CPZ in the putamen antagonized DA actions even after its local anesthetic effect had worn off.[118]

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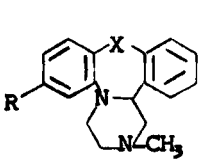
Chapter 2. Antidepressives and Stimulants

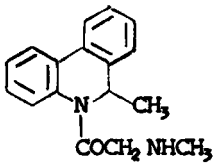
Carl Kaiser and Charles L. Zirkle
Smith Kline & French Laboratories, Philadelphia, Pa. 19101

Introduction - No new type of drug with clearly demonstrated utility in the treatment of depressive disorders emerged in 1972. However, reports that thyrotropin-releasing hormone (TRH) produced rapid improvement in a few depressed patients have generated considerable interest and publicity. Although research on the tricyclic antidepressives and amphetamine-like stimulants continues at a brisk pace, no major advances in the understanding of the actions of these drugs materialized. With the uncertain value of some animal tests for predicting antidepressive activity in man,[1] it is difficult to decide what experimental drugs, other than modifications of the standard tricyclics, should be included in the review. It is also sometimes difficult to tell from the limited data reported whether an agent is a potential antidepressive or is an amphetamine-like drug. So it is possible that we have either excluded some noteworthy compounds or have mislabeled others that have been included. A recent review on psychotropic drugs summarizes advances in antidepressive research.[2]

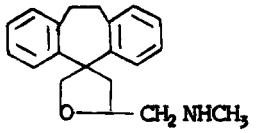
Tricyclic compounds with antidepressive activity - GC-94 (1a), GC-46 (1b), and GB-94 (1c, mianserin), which lack the pharmacological properties of antidepressives in animals, were selected for clinical study on the basis of their EEG effects in normal volunteers.[3] In depressed patients GC-94 had antidepressive efficacy equivalent to that of amitriptyline. Slight sedation was a side effect.[4] Mianserin (1c) was especially effective in endogenous depression.[5] A phenanthridine, OI-77 (2), also selected on the basis of quantitative EEG studies, was of benefit to acutely depressed patients.[6] In a series of tetrahydrofurfurylamines, a relative (3) of amitriptyline showed potency similar to that of desipramine (DMI) in tests for prevention of reserpine-induced ptosis in mice and tetrabenazine-induced depression in rats, but it was less effective in preventing oxotremorine-induced hypothermia in mice.[7] In a quantitative study of the anticholinergic action of several tricyclic antidepressives on an isolated rat fundal strip, GP 45437 (4) was the most potent.[8]



1 a) X=O; R=CH_3
b) X=O; R=H
c) X=CH_2 ; R=H



2



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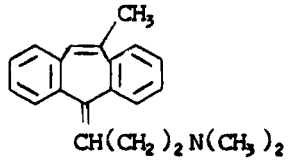
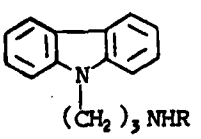


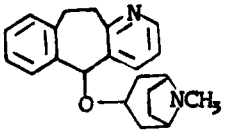
4

The potency of tricyclics as antidepressives and as inhibitors of catecholamine (CA) uptake by rabbit aortic[9] and rat cerebral cortical

strips[10] has been related to molecular conformational similarities to phenethylamines.[11] Like many antidepressives, two carbazoles 60-389a (5a) and 61-425 (5b), as well as several tricyclic tropanyl ethers, e.g. BS-7715 (6), inhibited uptake of CA by rat brain synaptosomes. The carbazole 5b was highly specific as an inhibitor of dopamine (DA) uptake.[12] A tricyclic-side chain bridged compound (7) inhibited norepinephrine (NE) uptake by rat vas deferens. Also, it caused increased motor activity (MA), but it did not prevent tetrabenazine-induced catalepsy in mice.[13]



5
a) R=H. b) R=CH_3



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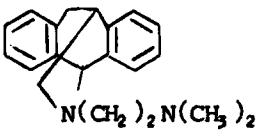

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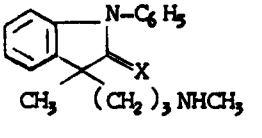
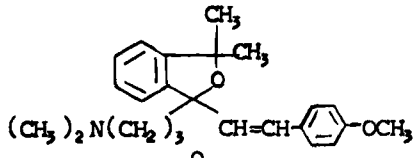


8 a) X=O. b) X=H_2

The metabolism[14] of tricyclic antidepressives and their interactions with other drugs[15] have been reviewed. A gas chromatographic method enabled accurate and specific quantitative determination of nortriptyline and some of its metabolites in human plasma and urine.[16]

Certain phenyl-substituted indolines, phthalans, phthalides and related bicyclics, which can be looked upon as ring-opened analogs of the tricyclic antidepressives, demonstrate antidepressive-like pharmacological activity. Amedalin (8a) and daledalin (8b) were the most potent of a series studied for potentiation of adrenergic mechanisms.[17] The phthalan 9 was the most effective (4X amitriptyline) of a series of styryl derivatives in a mouse test for tetrabenazine antagonism.[18] Antidepressive properties were also suggested for ICI 53,165 (10) which increased turnover of brain NE, but not DA or serotonin (5-HT), in rats.[19] Several 1,5-benzothiazepines related to the antidepressive thiazesim produced neuropharmacological actions similar to those of the parent. In rats, one of these (11) had selective antimuricidal activity (1.5X thiazesim).[20]



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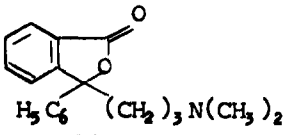

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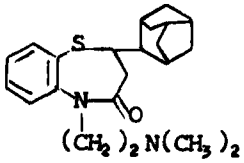
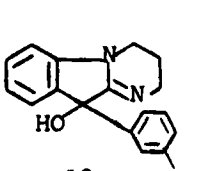


11

Other compounds with antidepressive activity - Several heterocyclic compounds have notable antidepressive-like pharmacological properties. Wy-23409 (12) reversed reserpine-induced hypothermia in mice and enhanced weight loss caused by methamphetamine in mice, yet it had little influence on MA and was not anticholinergic.[21] A related imidazo[1,2-c]-quinoline, AW-15'1129 (13) had typical antidepressive pharmacology with strong adrenergic and anti-REM effects. In clinical studies it increased the duration of non-REM sleep.[22] A γ-carboline, carbidin (14), is said to have both antidepressive- and neuroleptic-like properties in man and animals.[23] Imipramine-like activity, along with sedative and analgetic

effects, was noted for a series of pyridyl-substituted carbolines, e.g., PK 8 (15), which potentiated the action of dopa in pargyline-pretreated mice.[24] Related pentacyclic β-carboline derivatives, 16a and 16b, were equivalent to imipramine in this test, but were less effective in a mouse anti-reserpine test.[25] The tetracyclic carbazole 17, as well as its pyrazine ring-reduced analog, also displayed antidepressive-like pharmacology.[26] Both epimeric hexahydroindenopyridines 18, analogs of the antihistamine phenindamine, had an antidepressive profile similar to that of DMI in rats and mice - a finding which contradicts a postulate that a relationship between antidepressive and antihistaminic activities depends on the proximity of the basic and aromatic centers.[27]



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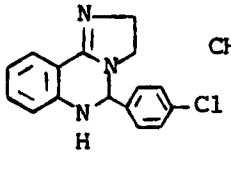

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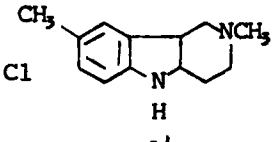

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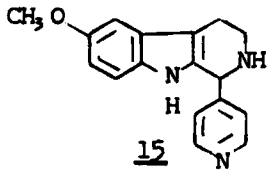

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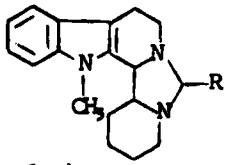
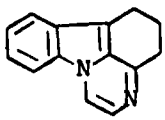


16 a) R=C_6H_5
b) R=C_2H_5



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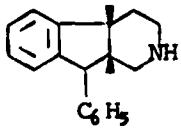
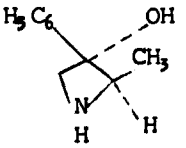


18

Several newly reported antidepressives are aralkylamines. Aletamine ($C_6H_5CH_2CH(NH_2)CH_2CH{=}CH_2$) presented a general pharmacological profile similar to that of the tricyclic antidepressives. It was more potent than imipramine in an anti-reserpine test and decreased spontaneous MA in mice.[28] In a study of ring- and side chain-substituted phenethylamines, 4-chlorophenethylamine was among the most potent; it inhibited uptake of NE and 5-HT by brain tissues (I_{50}=6.2-6.4μM).[29] Inhibition of NE uptake in rat vas deferns was examined for some conformationally-restricted phenethylamines. The cis (phenyl-CH_3)aziridine (19) was 7 times more potent than its geometrical isomer. Also trans-2-phenylcyclopropylamine (tranylcypromine) was 600 times more effective than its cis-isomer. These results suggest an anticlinal conformation of phenethylamines may be preferred for inhibition of NE uptake by both peripheral and CNS tissue.[30] Tetrahydropaveroline, a possible DA metabolite, antagonized reserpine and oxotremorine effects, but unlike many antidepressives it did not potentiate amphetamine responses in animals.[31] Another tetrahydroisoquinoline 20, one of a series, was equivalent to imipramine in preventing reserpine-induced ptosis in mice.[32]

Among a series of 2-phenethylpiperidinecarboxamides maximum potency in a modified dopa-potentiation test in mice was produced by 21 (ca. equipotent with amitriptyline).[33] The most potent of a series of aryloxypropanolamines was 22; it counteracted various reserpine-induced effects

at 5-10 mg/kg, i.p., and potentiated amphetamine-induced hyperactivity and pyrexia in mice and rats.[34] The most effective of a series of tropane-2β,3β-diol acetals was 23 which prevented (10 mg/kg, i.p.) and reversed (1 mg/kg, i.p.) reserpine-induced ptosis in rats.[35] Other aryloxyalkylamines with potential antidepressive activity included some basic ethers of 2-anilinobenzothiazoles, e.g. 24a, and benzoxazoles, e.g. 24b, which were potent (2X imipramine) reversers of reserpine-induced hypothermia in mice.[36] Antidepressive properties were also noted for ICI 58,834 (25). In mice this morpholine had anti-reserpine and anti-tetrabenazine effects comparable to those of DMI at 0.3-1.0 mg/kg, p.o.,[37] and it caused a rapid onset of antidepressive action in depressed patients.[38]



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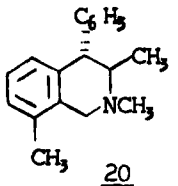

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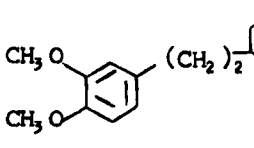

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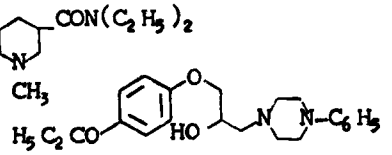

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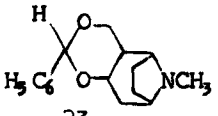

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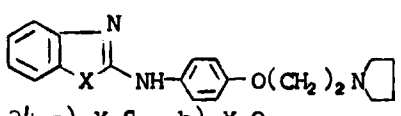
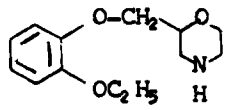


24 a) X=S. b) X=O



25

Several groups[39-41] presented clinical evidence that thyrotropin releasing hormone (TRH) may be useful in the treatment of depression. In small controlled studies a single injection of TRH produced prompt, but brief, improvement in depressed patients without causing undesirable side effects. Although the mechanism of TRH's antidepressive action is not understood, it potentiated the effects of dopa in both normal and hypophysectomized pargyline-pretreated mice. Thus, the potentiation is independent of the release of thyroid stimulating hormone from the pituitary gland.[42] An imipramine-triiodothyronine combination caused greater behavioral responses to L-dopa than either drug alone. Also, the combination, but neither compound alone, decreased adrenal tyrosine hydroxylase levels and caused marked hyperthermia in rats.[43]

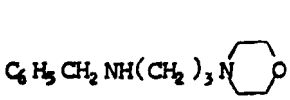
Rubidium and cesium salts produce antidepressive-like actions in animals. Unlike lithium salts, which decreased MA in mice, Rb and Cs caused increased MA when given s.c. for 7 days.[44] In rats, Cs increased 5-HT turnover without producing major NE and DA changes. In contrast, the main effect of Rb was a 300% increase in NE turnover. Additionally, Rb increased shock-induced aggression in rats, but Cs did not.[45] In a clinical study Rb (0.5-1.5 g/day for 20-86 days) improved 5 depressed patients. Although no serious toxicity was noted, the half-life (50-60 days) of Rb may present safety problems in its clinical use.[46]

With the discovery of several different forms of monoamine oxidase (MAO) having different substrate specificities, tissue distribution and

varied responses to inhibitors, interest has been rekindled in antidepressive drugs acting by a MAO-inhibitory mechanism. Such agents might be therapeutically-useful if peripheral and CNS actions can be separated. Of course, knowledge of the structure, function, kinetics, and mutual interactions of MAO's is important to progress in this area. Much of the present understanding has been summarized.[47-49] Although a number of reports describe new MAO-inhibitors, these are largely ones related to previously described chemical classes with this type of activity, and will not be reviewed here. The mode of interaction of pargyline and related compounds with MAO suggested that the flavin moiety of the enzyme is the target of the propynylamine-type of inhibitors.[50] In studies of tranylcypromine, the more potent MAO-inhibitory (+)-isomer was shown to have the absolute 1S:2R configuration. Thus, (+)-amphetamine and (+)-tranylcypromine have the same stereochemistry at the chiral center α to the amino group.[51] This is in contrast to the finding that (+)-amphetamine and (-)-tranylcypromine are the more potent inhibitors of NE reuptake in the CNS.[52]

Central stimulants - Pharmacological and stereochemical studies indicating that the effects of amphetamines on DA and NE may account for the major symptoms of amphetamine psychosis were reviewed.[53] To investigate the possibility that amphetamine might induce its diverse pharmacological effects by reacting with with various receptor sites in different conformations, the phenethylamine nucleus was incorporated into rigid and semi-rigid structures. The increased MA induced in mice by erythro-2-phenyl-3-aminobutanes, e.g. $C_6H_5CH(CH_3)CH(CH_3)NH_2$, and conformationally-related cis-2-phenylcyclohexylamine, suggested such an orientation might be required to cause this effect;[54] however, in a related trans decalin series even the isomers having this orientation were ineffective.[55]

Numerous new amphetamine derivatives have been reported; however, only those with novel structural differences from the prototype are reviewed here. Bridging of the α-methyl and amino groups, i.e. 2-benzylaziridine, resulted in loss of MA stimulating activity and a marked decrease (0.1X amphetamine) in anti-reserpine activity in mice.[56] A benzylamine 26 caused marked MA stimulation and shortened thiopental-induced sleeping time in mice.[57] Some 3-amino-2-phenylpropiophenones produced marked stimulant activity in mice; e.g., 27, caused this effect at 10 mg/kg, i.p.[58] Other CNS stimulants included the pemoline relative 28, which was effective in a mouse dopa-potentiating test,[59] and the sulfamide SaH 41-178 (29) which showed potent pentylenetetrazol-like properties in mice and cats.[60]



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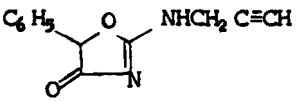

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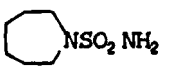

29

Biological observations and hypotheses - Various aspects of depressive illnesses and the putative neurotransmitters possibly involved in these disorders[1] have been reviewed and a modified "amine" hypothesis for affective illness has been advanced.[61] It is suggested that changes in

the balance between transmitter availability and receptor sensitivity are involved in depressions. Another postulate considers central cholinergic-adrenergic imbalances in depression and mania.[62] In a study of depressed patients, those who excreted the lesser quantities of 3-methoxy-4-hydroxyphenylglycol (MHPG) prior to antidepressive treatment had the best response to medication and excreted greater quantities of normetanephrine and MHPG, relative to the predrug period. Those patients who responded least well had a decrement in the excretion of these two metabolites of NE.[63] Clinical depressions which occurred following withdrawal of amphetamines after prolonged abuse were temporally associated with a decrease in excretion of MHPG; as the depressions subsided, MHPG excretion increased.[64] Clinical pharmacological and biochemical data have been reported supporting the postulate that neurotransmitter CA are functionally increased prior to the switch from depression to mania.[65] Although amitriptyline does not appear to have much effect on NE uptake or turnover in animals, a biochemical study in depressed patients indicated that it did cause changes in the metabolism of this amine.[66] Decreases in MHPG and vanillylmandelic acid (VMA) excretion were observed during amitriptyline treatment suggesting a decreased synthesis of NE in the brain as well as in peripheral sympathetic nerves. Increases were observed in the normetanephrine:MHPG and normetanephrine:VMA ratios suggesting the decrease in NE synthesis is accompanied by, possibly even caused by, a decrease in deamination of NE. In a trial lasting 4 weeks,[67] patients with primary depression responded equally well to imipramine (150 mg daily) or to L-tryptophan (9 g daily) Triiodothyronine enhanced the therapeutic response to imipramine but not to L-tryptophan. In cases of depression seen in general practice,[68] triiodothyronine also enhanced the therapeutic response to amitriptyline. Another study[69] failed to show an effect of triiodothyronine on the antidepressive activity of imipramine; the design of this study has been criticized (ref. 69, p. 55).

In rats, imipramine potentiated the increase in MA, but not the stereotyped behavior (SB), induced by L-dopa in combination with a decarboxylase inhibitor, an observation consistent with the fact that the antidepressant inhibits NE uptake but not DA uptake and the belief that SB is mediated by DA. Pretreatment, but not posttreatment, with imipramine and chlorimipramine obviated the L-dopa-induced 5-HT depletion. It is suggested that these tricyclic drugs may be useful in eliminating 5-HT mediated L-dopa effects.[70] Chlorimipramine was 6-10 times more potent in inhibiting 5-HT uptake than in inhibiting NE uptake in mouse brain *in vitro* and *in vivo*. Potentiation of L-dopa by the drug in mice was greater than expected from its inhibition of NE uptake.[71] The tricyclic antidepressant iprindole,[1] unlike DMI, altered neither the initial accumulation nor the metabolism of tritiated NE in brain or heart of the rat. Like the other tricyclics, iprindole increased brain levels of amphetamine (A) in the rat by inhibiting its p-hydroxylation.[72]

Although the SB induced by amphetamine (A) is generally believed to be mediated by DA, the roles of DA and NE in mediating the increase in locomotor activity (LA) induced by A are not yet clearly defined.[1] Coyle and Snyder[73] found that (+)-A was 10 times more potent than (-)-A in

inhibiting uptake of NE by telencephalic tissue of rats but that the two isomers were equipotent in inhibiting DA uptake by striatal tissue. These data have been used as bases for the interpretation of various experimental and clinical findings. Thus it has been proposed that: A-stimulated LA in rats is mediated by NE and A-induced SB is mediated by DA, based on respective 10:1 and 2:1 potency ratios of (+)- to (-)-A in eliciting these behaviors;[74] A-induced psychosis is DA-mediated since (+)- and (-)-A were about equipotent in eliciting psychosis;[75] in the hyperkinetic syndrome in children, aggression and hostility may be DA-related phenomena since (+)- and (-)-A were about equipotent in attenuating them, and anxiety and overactivity may be NE-related since (+)-A was much more effective than (-)-A in alleviating these symptoms.[76] In a study of intracerebral self-stimulation (ICSS) in rats, (+)- and (-)-A were about equipotent in enhancing ICSS when the stimulating electrodes were placed in the substantia nigra but (+)-A was much more potent than (-)-A when the electrodes were positioned in the medial forebrain bundle. It was suggested that A-enhanced ICSS in the latter situation was mediated to a large extent by NE.[77] However, other workers[78,79] have not duplicated the results of Coyle and Snyder. In one study,[79] the A isomers were found to be equipotent in inhibiting uptake of NE by telencephalic tissue whereas (+)-A was 5 times more potent than (-)-A in inhibiting DA uptake by striatal tissue. Certain details of other pharmacological and biochemical comparisons of the two isomers[80-82] also do not support the hypothesis that the relative potencies of (+)- and (-)-A in eliciting a behavioral effect may be an indication of the nature of the CA pathway involved. Thus caution should be exercised in the application of this concept. The possible involvement of CA pathways in minimal brain dysfunction (hyperkinetic syndrome) and the possible mechanism of action of A-like drugs in alleviating the symptoms of this disorder were discussed in a recent symposium.[83,84]

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Chapter 3. Narcotic Antagonists and Analgesics

Robert A. Hardy, Jr.
Lederle Laboratories, A Division of American Cyanamid Company, Pearl River, New York 10965

The 1972 literature is noteworthy for the lack of new analgesic agents being brought to advanced investigations including clinical trials. However, the possibility that improved narcotic antagonists may find better acceptance in treating post-addicts has sparked a marked resurgence of interest. Additional investigations of classical structures as well as studies of several interesting new systems are evident. Clinical trials of new antagonists are in progress. New methods of delivery and new combinations are being studied. More detailed examination of the mode of action of known agents, including pharmacokinetics and metabolism, is also evident.

Narcotic Antagonists - The resurgence in antagonist research[1] was marked by the symposium on Narcotic Addiction and Drug Abuse,[2] and the First International Conference on Narcotic Antagonists.[3] Legislation was enacted giving the Special Action Office for Drug Abuse Prevention special powers to coordinate resources on national problems of drug abuse, including research. This has also included government contracts for preclinical and clinical testing of promising candidates.

Current structure-activity information on compounds with "N-antagonist" substituents does not permit accurate prediction of predominant antagonist or agonist actions; structural differences are, at times, very subtle indeed. Consequently, both antagonists and analgesics are discussed together in the Structure-Activity Section.

The major disadvantages of present antagonists tried for supportive treatment of post-addicts are relatively short duration (e.g. naloxone), poor oral activity (compared to parenteral) and, at times, dysphoric effects when the doses are raised to gain greater duration or increased blocking effects (e.g. cyclazocine). An interesting approach to increasing the duration comprises incorporating the antagonist (ca 20% by wgt) homogeneously into a biodegradable poly-lactic acid polymer matrix and then inserting the blend into the body by surgery or injection.[4,5] Polymers containing cyclazocine, naloxone and (-)-BC-2605 (IIIa) are being studied. An alternative method is the use of insoluble salts for sustained-release actions.[6]

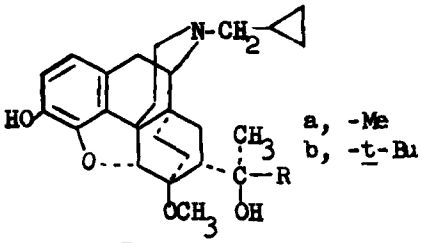
Another interesting direction in current antagonist research has been the incorporation of a small amount of naloxone with an orally active narcotic agent.[7,8] Naloxone alone is extremely potent in precipitating abstinence when given parenterally, but is 100-1000 times less potent orally. Thus, naloxone does not block an agonist when the mixture is given orally but prevents the euphoric effect of the mixed drug when subjected to parenteral abuse. Mixtures of naloxone with morphine, methadone and oxycodone have shown promising results in animals.

New Clinical Studies - Current reports do not include any new structures. Nalmexone (EN-1620A, N-dimethallyl-noroxymorphone), a mixed antagonist-analgesic, was compared to morphine for post-operative pain;[9] the equivalent dose was rather high, 70-90 mg intramuscularly. Naltrexone (EN-1639A, N-cyclopropylmethyl-noroxymorphone) in daily doses of 50-100 mg produced the same degree of antagonism as 4-8 mg of cyclazocine without side effects,[10] and is therefore a candidate as an antiabuse agent. Similar studies with (-)-BC-2605 have been initiated.[10] Levomethadyl acetate, a methadone-like agonist, was studied as a substitute for methadone maintenance;[11,12] its longer duration permitted 3 times per week administration instead of daily dosing.

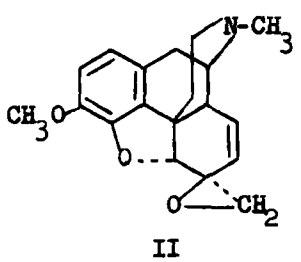
Additional trials with tilidine were reported,[13,14] as well as a continuing trial of propiram (ca 1/9 morphine im).[15] With the previously reported homopyrimidazole derivative MZ-144 (Probon) 300 mg orally equalled 30 mg of codeine;[16] the chemistry of this series was also reported in detail.[17] Etorphine (R&S 99-M), the high potency agonist in the 6,14-endo-ethenooripavine series, has been introduced in veterinary medicine for the immobilization of large animals; the anagonist diprenorphine (R&S 5050-M, Ia) is then used as the antidote.[18]

Structure-Activity; Agents under Investigation

Opiate Derivatives - Two compounds of the 6,14-endo-ethanooripavine series remain of special interest.[19] Diprenorphine (R&S 5050-M, Ia) is a pure antagonist more potent than naloxone and somewhat longer acting,[20] and is therefore a candidate for a non-addicting drug to block the euphoric effects of opiates.[1] The closely related buprenorphine (R&S 6029-M, Ib) is a mixed agonist (ca 70-90 x morphine)-antagonist with pentazocine-like physical dependence capacity in animals. In man it appears to have analgesic actions at low doses without dysphoric effects.[19] Additional chemistry of this series was published,[21] as well as additional agonist-antagonist data on newer compounds (esp. 7-oxo compounds).[22]



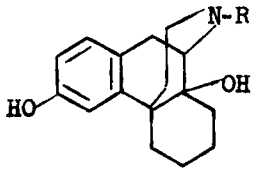
I



II

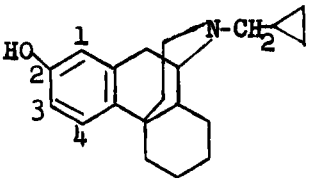
The new codeinone-6-oxirane (II) and the new 6-methylisocodeine obtained by reduction were synthesized; both had analgesic activity in the codeine range (mouse hot-plate).[23] The chemical synthesis of morphine-3-ethereal sulfate, its inactivity as an analgesic and its isolation from the urine of rats following administration of morphine was reported.[24]

Morphinans - The N-substituted-3,14-dihydroxymorphinans[25,26] are a new, fully synthetic series which parallel the 14-hydroxymorphinone series in their varied antagonist vs agonist properties. The principal activity is found in the (-)-isomers, as with other 3-hydroxymorphinans. (-)-BC-2605 (IIIa) is a potent antagonist (equiv to naloxone) with weak agonist properties. IIIb is a weaker antagonist (like cyclazocine), and IIIc is about 1/10 naloxone. With IIId and IIIe agonist properties predominate, each being more potent than morphine (mouse writhing); IIId is also an antagonist but IIIe is weak or inactive as an antagonist.[25]



a, $-CH_2$-cyclopropyl (-)
b, $-CH_2CH{=}CH_2$ (±)
c, $-CH_2C{\equiv}CH$ (-)
d, $-CH_2$-cyclobutyl (-)
e, $-CH_2CH{=}CMe_2$ (-)

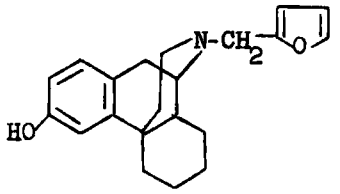
III



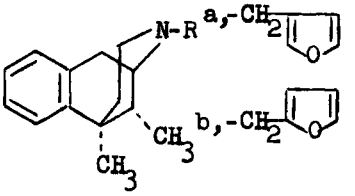
IV, (+)-isomer

The (+)-2-hydroxy derivative (IV) unexpectedly had antagonist properties, and represents an absolute configuration unique for an antagonist.[27] It did not suppress abstinence in morphine-dependent monkeys, and toxicity studies toward a possible clinical trial in man have been initiated. This series includes new morphinans oxygenated in the 1, 2, 3, or 4 position and with allyl, dimethallyl and cyclopropylmethyl N-substituents.

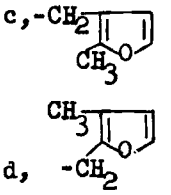
The N-furfuryl compound (V) is a weak antagonist (ca 1/4 nalorphine) with agonist properties also (ca 2/3 codeine, mouse hot-plate).[28] This represents a new series of N-substitutions which provides a wide range of antagonist to agonist properties (see VI).[29,30]



V, (-)-isomer



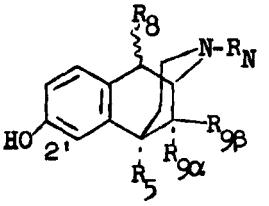
VI



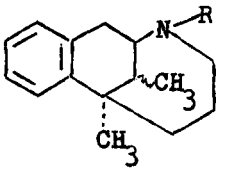
6,7-Benzomorphans - A new gradation of antagonist to agonist properties has been obtained by various N-furylmethyl substituents with both morphinans and benzomorphans.[29,31] VIa is a pure antagonist (ca 1/2 nalorphine). VIb is a mixed antagonist and weak agonist, and VIc is a strong agonist (ca 1/2 morphine, mouse hot-plate) with weak antagonist actions. VId is a pure agonist (ca 1/2 morphine), but does not suppress abstinence.[28]

Other new benzomorphans (VII) have included 9,9-dialkyl derivatives.[32] Introduction of a second methyl group in the 9α-monomethyl series (such

as phenazocine, cyclazocine, pentazocine, etc.) leads to compounds that are pharmacologically similar but more potent and longer acting. Direct introduction of oxygen on the benzylic carbon (VII, R_8) produces another subseries with a varied mix of agonist-antagonist properties.[33] The 8-oxo analog of cyclazocine (R_N=cyclopropylmethyl) is a potent agonist



VII

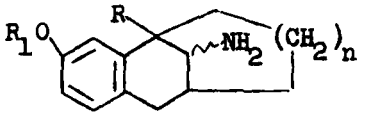


VIII

a, $-CH_2CH{=}CH_2$
b, $-CH_2CH{=}CMe_2$
c, $-CH_2$-cyclopropyl

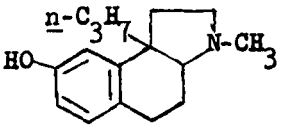
and weak antagonist. The 8- and 9-acetamido derivatives lacking the 2'-hydroxyl have also been prepared.[34] A modified synthesis of pentazocine has been described.[35] The N-methyl-homobenzomorphans with marked agonist activity have been extended to the N-substituted compounds VIII with antagonist activity.[36]

Bridged-aminotetralins, Benzazepines and Benzazocines - Several additional modifications of the rigid analgetic benzomorphans are providing interesting new leads. The common theme in these families is modification (or elimination) of the piperidine ring but maintaining a 2-carbon unit between the aromatic ring and the basic nitrogen together with bulk



IX

IXa, R_1 = Me, R = Me, n = 2
b, R_1 = H, R = Me, n = 2
c, R_1 = H, R = Et, n = 2
d, R_1 = Me, R = Me, n = 3
e, R_1 = H, R = Me, n = 3

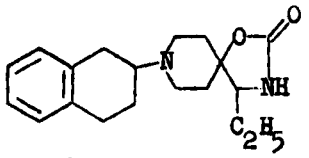


X

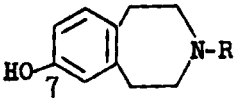
and/or favorable steric factors provided by bridged rings or by 7- and 8-membered rings containing the nitrogen. Some of the new 1,3-bridged-2-aminotetralins (IX)[37-39] were more potent than morphine (rat tail-flick) and also possessed antagonist actions. A 5-carbon bridge was optimal, hydroxy substitution gave greater activity than methoxy on the aromatic ring, and ethyl at the bridgehead carbon gave greater analgesic potency than methyl. The analgesic ED_{50}'s of IXc and IXe were 0.25 and 0.47 mg/kg (im), respectively.[38]

TA-404 (X) shows similar elements of structure and was the most potent of its series.[40] It was about 1/3 morphine (mouse hot-plate) and did not suppress abstinence in morphine-dependent monkeys.[28] An independent study of this series as analogs of profadol (see XV) was also reported.[41] The 1-(2-tetralyl)-4-spiropiperidine XI was a potent adrenolytic, but also was an analgesic ca 8-10 times codeine (writhing; best of

series).[42] The adrenolytic activity appeared to be equal to or greater than the analgesic activity; this compound was selected for clinical trials. Synthesis of simpler tetrahydro-2-naphthylamines for analgesic purposes has also continued.[43]



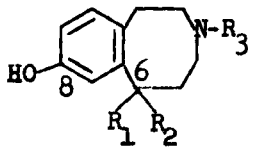
XI



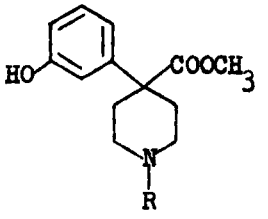
a, $-CH_2CH=CH_2$
b, $-CH_2CH=CMe_2$

XII

The tetrahydro-1H-3-benzazepines[44] represent another modification of the tricyclic benzomorphans. A large number of 7-hydroxy (XII) and 7-methoxy derivatives were examined for analgesic and antagonist actions;[45] compounds with hydrogen, methyl, ethyl, propyl or cyclopropylmethyl groups on nitrogen were weak or inactive. However, XIIa and XIIb had antagonist actions in the nalorphine range depending on the test method and route of administration. They did not show analgesic activity and, thus, resembled the pure antagonist actions of naloxone. Similarly, an extension of previous work on 3-benzazocines which had poor analgesic activity has given a new class of 6-substituted derivatives XIII as potent analgesics[46]



XIII

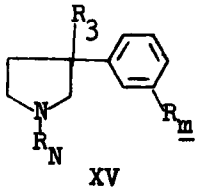


a, $-CH_2$-(thienyl)
b, $-CH_2$-(2-methylfuryl)

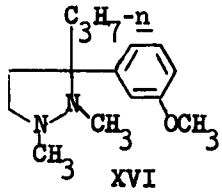
XIV

Piperidines and Pyrrolidines - Previous conclusions that antagonists are not readily found by N-substitution in the 4-phenylpiperidine series are being modified. Antagonists are now being obtained with a phenolic m-hydroxyl and suitable N-substituents.[47] XIVa was a weak analgesic (ca 1/3 codeine, mouse hot-plate) that did not suppress abstinence, while XIVb was a potent agonist (ca 1/2 morphine) with complete suppression.[28]

In the pyrrolidine series which includes the mixed agonist-antagonist profadol (XV; R_N=Me, R_3=n-Pr, R_m=OH) additional studies with branched-3-alkyls and longer N-alkyls provided no improvement.[48,49] The N-allyl and N-cyclopropylmethyl derivatives (R_3=Me, R_m=OH) are now being explored as selective antagonists.[50] A series of pyrazolidines[51] was modeled after the pyrrolidine analgesics, and XVI, the best compound, was an analgesic (ca 2/3 codeine, mouse hot-plate). Six-membered piperidazine analogs appeared to be weak or inactive.[51]

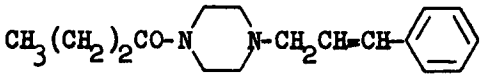


XV

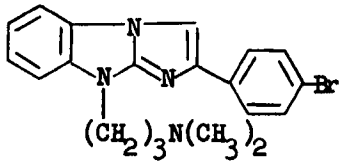


XVI

Miscellaneous Compounds - Stereochemical studies and the absolute configuration of the (+)- and (-)-isomers of tilidine were reported.[52] The piperazine derivative AP-237 (XVII) was more effective than morphine or pentazocine in the rat inflamed-paw pressure test.[53] AP-237 was found to be clinically effective in Japan (1970).[53] The (S)-optical isomer of 1-cyclohexyl-4-(1,2-diphenylethyl)piperazine was reported to have morphine-like analgetic activity.[54]



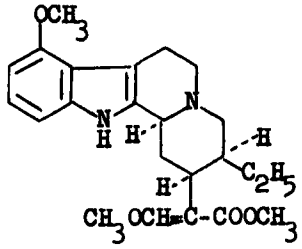
XVII



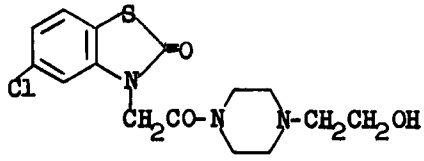
XVIII

A series of imidazolo-benzimidazole derivatives[55] was studied as an extension of the older 2-benzyl-1-(dialkylaminoalkyl)benzimidazoles. XVIII was the most effective compound; analgesic ED_{50}'s (po) were 6.0 (mouse writhing) and 9.0 mg/kg (mouse hot-plate).

A detailed pharmacological evaluation of the indole alkaloid mitragynine (SKF-12,711, XIX), isolated from the leaves of the Malayan tree Mitragyna speciosa, showed oral analgesic activity slightly less that of codeine with minimal side effects.[56] Poor subcutaneous activity suggested the oral activity might be due to a metabolite.



XIX



XX

Antiinflammatory Analgesics - This class of compounds is discussed in Chapter 22. Details of analgesic testing were reported for several anti-inflammatory compounds. Tiaramide (NTA-194, XX) was about 1/2-1/5 the potency of codeine in 3 analgesic procedures (rat tail-flick, rat tail-pinch, mouse writhing) and was a stronger antipyretic than phenylbutazone.[57]

The recent interest in analgesic 4-quinazolinones[28] has continued, but current emphasis appears to be on 2-quinazolinones which are predominantly antiinflammatory.[58,59] 1-Cyclopropylmethyl-6-methoxyquinazolin-2(1H)-one (SL 573) had 2-4 times the analgesic activity of mefenamic acid and was about equal to phenylbutazone (mouse writhing, rat paw-pain).[58]

Pharmacology, Biochemistry and Metabolism - A variety of present studies are being directed toward a better understanding of both analgesic and antagonist actions; the following selected examples are illustrative.

The Nilsen method (mouse, electrical stimulation to the tail) appears to be equal or superior to present tests, including mouse writhing, for detecting analgesia in compounds having dual agonist-antagonist actions.[60] Naloxone applied to discrete brain regions of morphine-dependent rats showed marked differences in ability to precipitate severe abstinence suggesting that the sites of adaptation to morphine have neuroanatomical specificity.[61] Cyclic AMP antagonized the effects of morphine in naive mice, and repeated administration increased the rate of development of tolerance and appeared to enhance the withdrawal response (jumping) precipitated by naloxone.[62] Competition for specific binding of tritiated naloxone to rat brain homogenates by various agonists and antagonists, which reduced the binding, closely paralleled their pharmacological potency.[63]

Several new aspects of methadone metabolism and pharmacokinetics were reported. The N-oxide was found in the urine from addicts on methadone treatment.[64] The time course of methadone in the plasma of subjects receiving methadone maintenance was studied.[65] Stereoselectivity and differential metabolism of (+)- and (-)-methadone (^{3}H) suggested the formation of an active metabolite in rat brain from the (-)-isomer but not from the (+)-isomer.[66] α-(-)-Methadol and α-(-)-N-demethylmethadol are metabolites of (+)-methadone, and are potent analgesics.[67]

Metabolism and distribution of racemic and optically active ^{3}H-prodines were investigated in mice; the results suggested the large potency differences between isomers were primarily due to stereoselective events at the CNS receptors and not to differences in metabolism.[68] The physiologic disposition of ^{3}H-alphaprodine was also studied in dogs.[69]

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Chapter 4. Sedatives, Hypnotics, Anticonvulsants and General Anesthetics

A. D. Rudzik and W. Friis
The Upjohn Company, Kalamazoo, Michigan 49001

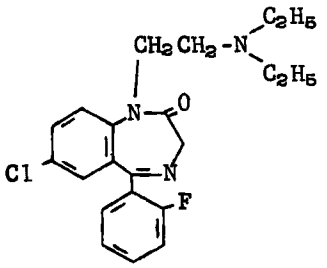
Introduction - During the last year there has been a proliferation of reports of compounds possessing depressant activity. Some of the compounds will be considered in other chapters, but because of reports of sedative-hypnotic and anticonvulsant activity in certain compounds, they may also be covered in this section. This is especially true in the case of the benzodiazepines where it is difficult to separate antianxiety from sedative-hypnotic activity. An attempt was made to cover those compounds where more than preliminary screening data is available or where novel structural types of compounds were presented.

Sedatives and Hypnotics - Insomnia is among the most common disorders dealt with in medical practice and despite much research work in recent years, sleep and insomnia are not well understood. The causes of insomnia and its management have recently been reviewed by Johns.[1] In his review, he describes both the intrinsic and extrinsic factors which can cause insomnia and the practical aspects of its treatment.

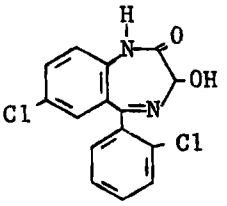
In an effort to better understand sleep and the effects of hypnotic drugs on sleep patterns, numerous studies have been undertaken to evaluate the effect of drugs on polygraphically monitored sleep in man. These studies have been evaluated critically by Freemon.[2] He describes the way that sleep laboratory studies are done, the division of sleep into its various stages, and the numerous pitfalls in this kind of research. The review by Freemon includes a summary of the results obtained in 65 different studies with both new experimental compounds and currently available hypnotic and central nervous system drugs.

The clinical choice of sedative-hypnotic drugs for the management of insomnia has also been reviewed recently by Greenblatt and Shader.[3] The authors review the clinical conditions in which hypnotics should be used as well as the advantages and disadvantages of currently available hypnotic agents. They conclude that current evidence favors benzodiazepine derivatives since suicide is virtually impossible with them and they do not interact with oral anticoagulants.

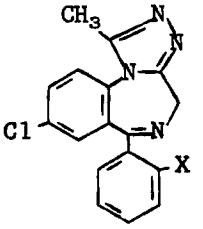
A characteristic of most benzodiazepines is their propensity to produce sedation and hypnosis in both animals and man. Numerous papers have appeared in the last year describing the hypnotic activity of flurazepam (I) in man.[4,5,6] The effect of various hypnotics on performance (vigilance, eye-hand coordination, cognitive-association and decision making) was reported by Bixler *et al*[6] who found that in general secobarbital produced a more consistent and greater decrement in performance than flurazepam.



I



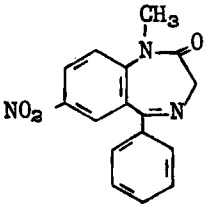
II



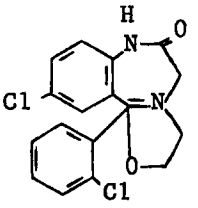
III - X=H
IV - X=Cl

Sleep laboratory studies on lorazepam (WY 4036, II) have shown that this compound depresses REM sleep but there is no rebound increase in REM during the post-drug nights. Stage 4 sleep was not decreased by lorazepam.[7] No changes were seen in clinical laboratory studies or physical examinations.

The potent activity of the triazolobenzodiazepines (U-31,889, III and U-33,030, IV) has been summarized.[8] The effect of one of these derivatives, compound (III) in sleep laboratory studies has been reported by Itil et al[9] who conclude that compound III could be useful in chronic sleep disturbances and in patients with nightmares, somnambulance and night terrors.



V



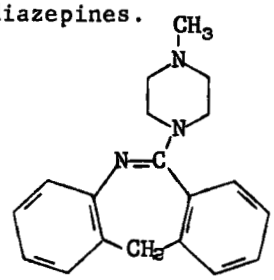
VI

Compound V (S-1530), the 1-methyl derivative of nitrazepam, a marketed hypnotic agent, has been shown to have both antianxiety and hypnotic activity clinically.[10] In animals compound V was found to be more potent than either nitrazepam or diazepam as a muscle relaxant, anticonvulsant and "sleep inducer".[11]

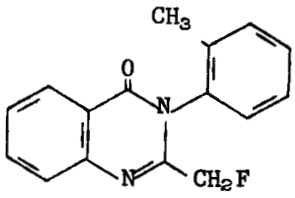
Compound VI (CS-370) was found in the process of screening derivatives of the benzodiazepine derivative, oxazolam. This compound is more potent than oxazolam in laboratory tests and has a lower toxicity than does diazepam.[12]

The animal pharmacology of perlapine (VII) has recently been reviewed and the compound has been postulated to differ in mechanism of action from other sleep promoting agents.[13] Moreover, the compound

differs pharmacologically from potent neuroleptics and also from the benzodiazepines.



VII

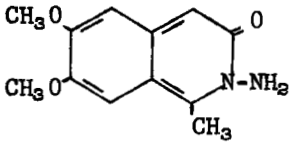


VIII

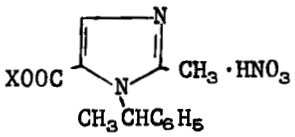
In sleep laboratory studies in man, compound VII caused a reduction in REM sleep but did not reduce stage 3 or 4 sleep.[14] This was interpreted as advantageous since stage 3 and 4 sleep can be linked to the restorative properties of sleep.

The 2-monofluoromethyl analog of methaqualone (VIII - HQ-355) has been reported to be a more potent hypnotic agent than methaqualone with a lower toxicity.[15] Compound VIII also possessed more potent anticonvulsant activity.

K. Nagarajan et al[16] have reported the CNS depressant activity of a series of derivatives of benzodiazepinones. One of the derivatives, compound IX, showed dose related sedation, ptosis and ataxia. The compounds had low toxicity.

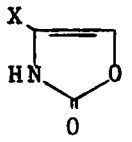


IX

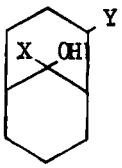


X - X=CH_3
XI - X=C_2H_5

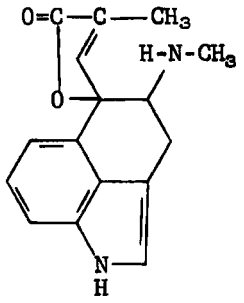
The methylimidazole derivatives (X, XI) resemble general anesthetics, as doses causing loss of righting reflex lie close to those affecting loss of other reflexes.[17] At non-hypnotic doses, the compounds (X, XI) potentiated the hypnotic effect of sodium phenobarbital.



XII X=2-Naphthyl
XIII X=1-Naphthyl



XIV X=C_6H_5
Y=4-Phenyl-1-piperazinyl



XV

Two oxazolinone derivatives (XII, XIII) were found to exhibit sedation and muscle relaxation.[18] Compound XII was also found to potentiate the hypnotic effect of alcohol. This was surprising for this series of compounds because most other derivatives antagonized barbiturate-induced sleep.

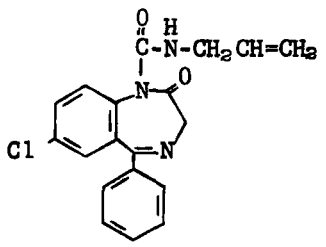
The most interesting series of compounds possessing CNS depressant activity are the bicyclononanol derivatives.[19] Compound XIV, the most active of the series, is equivalent in activity to chlordiazepoxide on all endpoints except antagonism of pentylenetetrazol-induced clonic convulsions. The compound is also much less toxic than chlordiazepoxide.

Finally, pharmacological studies with a new indole alkaloid, rugulovasine (XV), have shown that the alkaloid depressed spontaneous motor activity, prolonged the duration of loss of righting reflex caused by barbiturate, and enhanced the similar potentiating effect of reserpine in mice.[20]

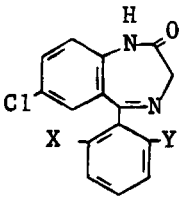
Anticonvulsants - Although primidone has been in clinical use since 1952, little work has been done on its metabolism in man. Baumel et al[21] found that after a single oral dose of primidone in man, PEMA (phenylethylmalonamide) appeared in the serum, whereas phenobarbital was not detected. This finding differs from previous studies in animals where administration of primidone results in the production of PEMA as well as phenobarbital. In epileptic subjects receiving primidone chronically, however, both PEMA and phenobarbital accumulated in the serum. PEMA was found to have anticonvulsant activity in rats and also potentiated the anticonvulsant activity of phenobarbital. These results correlate with the recent evidence from animal studies that the total anticonvulsant action of primidone exceeds that attributable to phenobarbital alone.

One of the properties of many central nervous system depressant drugs is their ability to antagonize seizures produced by convulsant agents or electroshock. The benzodiazepine derivatives are especially active in this regard with pronounced activity against chemically induced

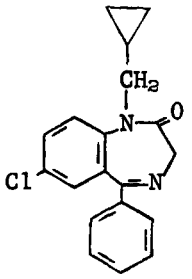
seizures in animals but with less activity against electroshock seizures.



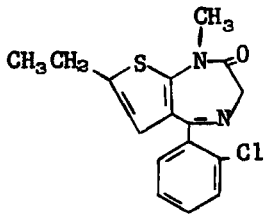
XVI



XVII X=F;Y=F
XVIII X=F;Y=H



XIX



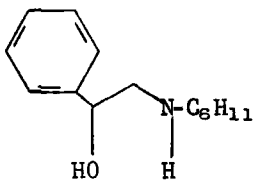
XX

The 1-allylcarbamoyl benzodiazepine (XVI) is equipotent to diazepam in antagonizing convulsions induced by nicotine, thiosemicarbazide, strychnine and pentylenetetrazol and electroshock.[21] The ED50 value to antagonize the chemically induced convulsions is significantly lower than that required to antagonize maximal electroshock-induced seizures.

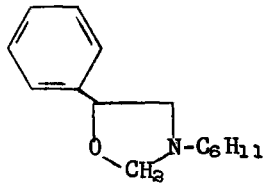
The 2,6-difluorobenzodiazepinone (XVII) also possesses potent anticonvulsant activity against chemically induced convulsions.[22] It is approximately four to ten times as active as diazepam in this regard. The monofluoro derivative (XVIII) is at least as active as the difluoro derivative (XVII) as an anticonvulsant.

The pharmacological properties of prazepam (XIX) have previously been published,[23] however, a more extensive study by Boissier et al[24] shows that the compound has potent anticonvulsant activity with low sedative action, a wide margin of safety, and a long duration of activity.

A thienodiazepine derivative (XX) is two to three times more active than diazepam and six to nine times more active than chlordiazepoxide in antagonizing pentylenetetrazol and bemegride induced convulsions in mice.[25] The compound has been tested clinically in preoperative sleep disturbances and found to be very effective.[26]

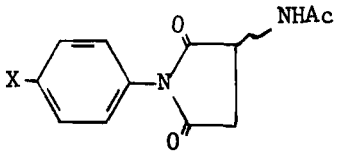


XXI

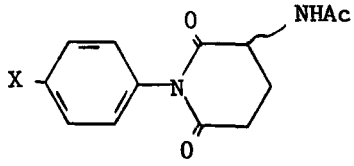


XXII

2-Cyclohexylamino-1-phenylethanol (XXI) has been found to potentiate the hypnotic effect of pentobarbital and to markedly antagonize convulsions produced by pentylenetetrazol but not electroshock or strychnine.[27] Cyclic compounds (XXII) derived from XXI showed less activity under the same test conditions.

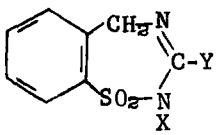


XXIII X=H
XXIV X=Cl

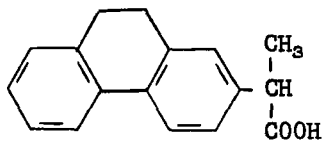


XXV X=H
XXVI X=Cl
XXVII X=NO_2

The stereo structure-activity relationships of a series of acetyl-D(R) and L(S)-N- succinimides (XXIII, XXIV) and glutarimides (XXV, XXVI, XXVII) were studied.[28] Succinimides and glutarimides having the D(R) configuration exhibit activity equal to or greater than the activity exhibited with the L(S) anticonvulsants. Compounds XXIII, XXIV, XXV, XXVI and XXVII have activity which compares favorably with the activity of drugs of clinical significance.



XXVIII X=nC_6H_{13}
Y=nC_4H_9



XXIX

Fernández-Tomé et al[29] have reported on the synthesis of a series of 2,5 dihydro-1,2,4 benzothiadiazepine 1,1-dioxides of which compound XXVIII is representative. Compound XXVIII antagonizes maximal electroshock seizures in mice but has weaker activity against pentylenetetrazole and strychnine induced convulsions. The toxicity of this compound is extremely low (LD50 >2000 mg/kg, i.p.).

Compound XXIX produces the same degree of CNS depression as phenobarbital or chlordiazepoxide and possesses anticonvulsant activity against maximal electroshock induced convulsions.[30]

General Anesthetics - The pharmacological properties of CT 1341 (Althesin), a steroidal anesthetic agent which contains alphaxalone and alphalolone acetate, have recently been described.[31] It is a potent intravenous anesthetic in animals which produces rapid induction of anesthesia without vascular irritation. Initial trials of CT 1341 in man appear to substantiate the finding in animals.[32] In further anesthetic, cardiovascular and respiratory studies in animals, it was found that CT 1341 has a wider therapeutic latitude, produces less respiratory depression, and has greater efficacy than currently used intravenous anesthetics.[33]

Thirty-four halogenated methyl ethyl ethers were evaluated as volatile general anesthetics by Terrell et al.[34] Twelve of the compounds had good anesthetic properties in mice. Compounds XXX and XXXI are currently undergoing clinical trial.

$F_2HCOCHClCF_3$ F_2HCOCF_2CHClF

XXX XXXI

An additional series of methyl pentahaloethyl and methyl heptahaloisopropyl ethers were evaluated as anesthetic agents.[35] Both sedation and anesthesia were observed but the potency in general was diminished when compared to the halogenated methyl ethyl ethers.

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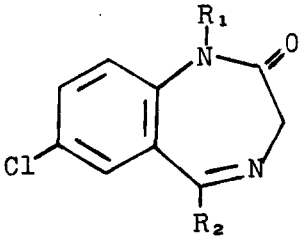
Chapter 5. Skeletal Muscle Relaxants*

Robert C. Landes, Roger J. Stopkie, ICI America Inc., Wilmington, Delaware, and Vincent T. Spaziano, Dept. of Chemistry, St. Louis University, St. Louis, Missouri

This report covers what were judged to be the more significant contributions to the field during the period 1968-1972. Three reviews of general interest in this area appeared.[1-3] The skeletal muscle relaxant (SMR) activity of 1,4-benzodiazepines was recently summarized.[4]

The distinction between centrally and peripherally acting SMR agents has become blurred with the discovery that several of the classical "centrally acting" drugs have demonstrable, perhaps clinically significant effects on peripheral structures. For example, mephenesin and chlormezanone prolong the refractory period of the neuromuscular junction.[5]

The 1,4-benzodiazepine, prazepam (I), in a double-blind clinical trial, was found orally effective in reducing skeletal muscle hypertonia in 9 of 14 multiple sclerosis patients.[6]

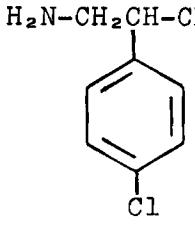


	R_1	R_2
I	CH_2-cyclopropyl	C_6H_5
II	CH_3	

Clinical studies relating to the use of tetrazepam (4261 CB, II) report varying degrees of effectiveness in relieving spasticity. In one study,[7] patients with skeletal muscle hypertonia benefited from 50-300 mg/day of the drug, while in a double-blind clinical trial with children having spastic cerebral palsy, the drug was ineffective.[8]

Recent studies have examined further the nature of the SMR activity of diazepam.[9,10] In cats, diazepam may exert its depressant effects on spinal reflexes by enhancing presynaptic inhibition.[11,12] The action appears to be independent of any effects on supraspinal structures.[13]

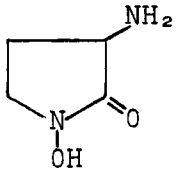
*Dedicated to the memory of the late Dr. Hugh B. Donahoe, Professor of Chemistry, St. Louis University, who was one of the original authors.



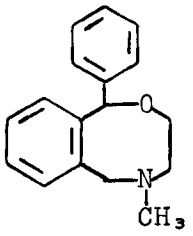
III

Baclofen (Ciba 34647-Ba, III), a derivative of the inhibitory CNS amino acid γ-aminobutyric acid (GABA) has been shown, in double-blind controlled studies, to be an effective anti-spasticity agent in the oral dose range 30-100 mg/day.[14-16] Side effects are reported to include nausea and muscular weakness. The site of action may be localized in the area of the spinal cord made up of afferent nerves going from muscle spindles to α-motoneurons.[17]

1-Hydroxy-3-aminopyrrolidone-2 (HA-966, IV) which structurally resembles the cyclic form of GABA, elevates the threshold to strychnine convulsions in mice.[18] It abolishes the polysynaptic flexor reflex in cats possibly by a depression of central interneurons. The delayed action of HA-966 is consistent with the proposal that it undergoes metabolic conversion to an active form, possibly GABA or γ-hydroxybutyric acid.

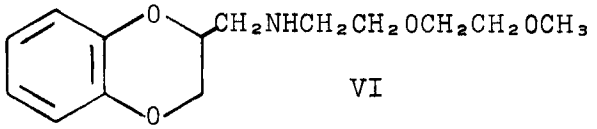


IV



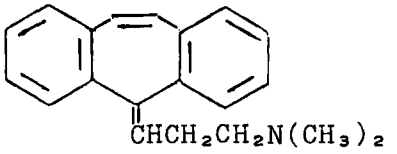
V

Nefopam (Riker 738, V), a cyclic analog of diphenhydramine, has significantly less antihistamine and anticholinergic activity than the parent molecule.[19] In cats, nefopam blocked patellar reflex facilitation induced by stimulation of the reticular formation. A double-blind study of 24 patients showed nefopam to be superior to placebo in relieving pain, tenderness, and muscle spasm.[20]



VI

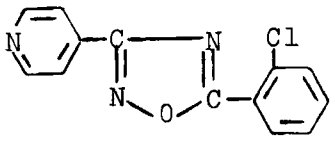
Ambenoxan (VI) reduces decerebrate rigidity in rabbits.[21] It also produces skeletal muscle flaccidity without loss of the righting reflex when administered orally or parenterally to rats, rabbits, dogs, and monkeys. The authors indicate that in preliminary human studies, a muscle relaxant effect was observed with intravenous or oral doses.



VII

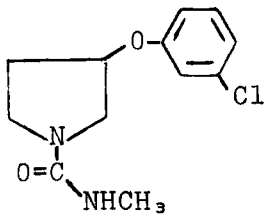
It has been reported that cyclobenzaprine (MK-130, VII), a tricyclic amine related to amitriptyline is effective orally in the treatment of spasticity at 30-70 mg/day.[22] However, in a double-blind crossover trial, it was ineffective at 60 mg/day.[23]

RJ-64 (VIII), an oxadiazole, acts in a manner similar to chlorzoxazone in animal studies.[24] In rats, RJ-64 proved to be seven times more potent than chlorzoxazone with regard to antistrychnine activity. In a subsequent structure-activity study involving thirty-five 1,2,4-oxadiazoles,[25] none was found substantially more effective than VIII.

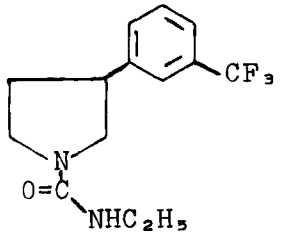


VIII

AHR-2666 (IX), exhibits both central and peripheral effects, blocking cat spinal interneurons, and directly depressing skeletal muscle.[26] In spite of these combined actions, no impairment of respiration was seen.



IX

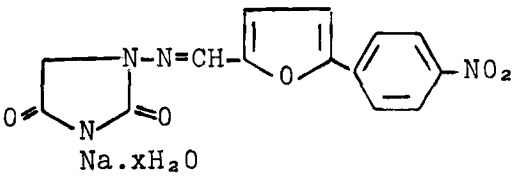


X

A structurally related compound, AHR-2776 (X), selectively blocked cat polysynaptic reflexes, indicating no direct depressant effects on muscle.[27] AHR-2776 also inhibited the effects of reticular formation stimulation on the patellar reflex.

The α-adrenergic blocker thymoxamine, 5-(2-dimethylaminoethoxy)carvacrol acetate, was reported to produce a transient reduction in spasticity in a group of patients with lower limb spasticity.[28] In normal volunteers, using a double-blind technique, this drug reduced the ankle jerk approximately 34% at an intravenous dose of 0.1 mg/kg. It was suggested that thymoxamine depresses fusimotor activity as a result of action at nor-adrenergic receptors in the spinal cord.[28,29]

The amino-hydantoin dantrolene sodium (XI), originally suggested as acting mainly via the CNS[30] has subsequently been found to exert its major action peripherally, possibly on the excitation-contraction coupling mechanism of skeletal muscle.[31] Double-blind clinical studies have demonstrated the effectiveness of dantrolene in relieving human spasticity.[32,33] Side effects were minimal and included drowsiness, weakness, and "drunk" feelings. Most of these effects disappeared during the first week of treatment.



XI

While drug treatment of spasticity remains a difficult problem, this report indicates several new, interesting and promising approaches to the development of more effective muscle relaxants.

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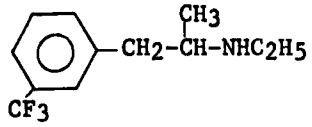
Chapter 6. Agents Affecting Appetite

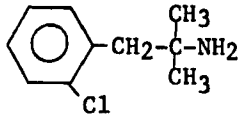
George C. Heil and Stephen T. Ross
Smith Kline & French Laboratories, Philadelphia, PA 19101

Introduction - The changes in the area of anorectic drugs and the treatment of obesity in the past three years, although of major importance, have centered around medical opinion and legislative activities rather than in breakthroughs in research. Severe criticism of the use of appetite suppressant drugs has emerged from government and medical sources[1] and tight regulation of the manufacture, sale and promotion of the amphetamine type anorectics has resulted.[2] The Food and Drug Administration has placed the amphetamines into BNDD's Schedule II (the most closely regulated category other than narcotics) because of their abuse potential. Medical opinion about the usefulness of appetite suppressants varies from negative[3] to strongly positive.[4,5] The result of all this controversy has been a critical assessment of the treatment of obesity and the use of anorectics in that treatment. A meeting on Drugs and the Control of Overweight[6] brought together a number of experts in the field and resulted in the development of an authoritative medical position on the topic. The clinical efficacy of anorectics as adjunctive agents in the treatment of overweight was supported by these experts.[7] The general overview was that the agents were useful but more rational short term use of anorectics was indicated and that drugs have a place in the treatment of overweight. They should not, however, be used as the sole means of treatment.

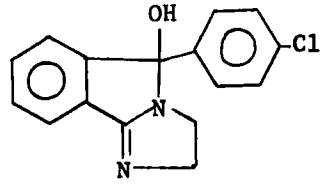
Three new drugs for the treatment of obesity in the U.S. have emerged during the past three years, fenfluramine (I), clortermine (II)[8] and mazindol (III).[9,10,11] Although used abroad for some time, NDA's for these drugs are expected to be approved shortly.[2,12] Fenfluramine will be placed in BNDD Schedule IV (least restrictive category) and clortermine and mazindol will probably join diethylpropion, chlorphentermine and benzphetamine in Schedule III.[12]



I



II



III

Fenfluramine - The anorectic that received the most attention in the past three years was fenfluramine. Several reviews of fenfluramine's activities were presented at an International Symposium on Amphetamines and Related Compounds at Milan and were subsequently published in a single volume.[13] This volume represents the "state of the art" with respect to amphetamine and related substances and will surely be a source of reference for some time to come.

In animals, fenfluramine has been shown to be slightly less potent as an anorectic than d-amphetamine but with a longer duration of action.[14-16] Its effect on the CNS has been studied and its activities include decreased confinement motor activity in rats[16] and decreased spontaneous motor activity in mice[14], prolongation of pentobarbital sleep time in mice[17] and diminution of hyperactivity caused by amphetamine.[17] Fenfluramine has been reported to antagonize the toxicity of amphetamine in aggregated mice but not in isolated mice[13x] and is also capable of reducing isolation-induced aggressive behavior.[18] E.E.G. studies in Rhesus monkeys indicated that fenfluramine produces activation of the ventromedial hypothalamus (satiety center) and deactivation of the lateral hypothalamus (feeding center) along with a general slowing of cortical waves.[19] Fenfluramine's slight hypotensive effect in anesthetized animals has been documented.[20,21] A study of the effects of fenfluramine on the autonomic nervous system reported that, depending on dose and preparation used, fenfluramine both facilitated and inhibited the responses elicited by sympathetic stimulation of isolated and intact animal preparations.[22] Fenfluramine has also been shown in animals to produce certain pharmacological effects resembling imipramine.[23] In rats, fenfluramine was found to considerably reduce the para-hydroxylation of amphetamine and accelerate amphetamine's side chain metabolism.[24]

Biochemical studies revealed that fenfluramine decreased the content of norepinephrine and dopamine in mouse and rat brain and pretreatment with a monoamine oxidase inhibitor reversed the effect of fenfluramine from decreasing to increasing motor activity.[25] Additional reports indicate that fenfluramine and amphetamine differ in the mechanism by which they release norepinephrine from the brain.[26] Several investigators reported that fenfluramine caused depletion of 5-hydroxytryptamine in rat brain.[27,28] Pharmacological studies with synthesis inhibitors of neurotransmitters and antagonists led to the suggestions that the anorectic effect of fenfluramine might be due to release of brain serotonin[29,30] or to stimulation of tryptaminergic neurons directly.[28]

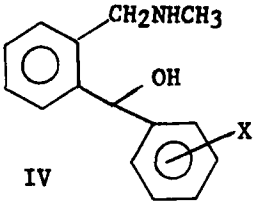
Fenfluramine was also shown to inhibit the lipolytic response induced by pancreatic lipase *in vitro*[31] and to produce a difference in ketone body metabolism during treatment compared to the effect of starvation suggesting an effect upon enzymes involved in ketone-body metabolism.[32] Other studies in rats reported inhibition of the uptake of oral ^{14}C-glucose by adipose tissue, decreased epididymal fat pad weight and plasma triglycerides produced by fenfluramine.[33] In studies with high fat diet fed rats, however, the loss in body weight produced over 5 days with fenfluramine and d-amphetamine could be attributed to the degree of anorexia produced.[34] Clinical results with fenfluramine indicated its efficacy as a treatment for refractory obesity even without dietary restriction[35] and in general that it is an effective and safe drug for use in a weight loss program.[13g,b] It has been reported, however, to produce depression of mood in some patients after abrupt cessation of treatment.[36]

Research Compounds - Research on new antiobesity agents may be divided into three categories: (1) those compounds distinctly outside the amphet-

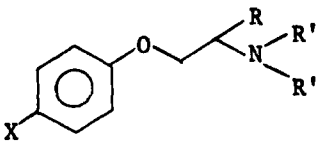
amine structural classification, (2) compounds containing the amphetamine structural skeleton, and (3) N-substituted derivatives of amphetamine itself. The liability of amphetamine and many of its congeners for abuse of its stimulant properties and tolerance development to its anorectic properties continues to apply pressure on drug research teams to find safe anorectic agents outside this structural and pharmacological type entirely. Screening programs have produced new leads. These compounds tend to be of high to moderate potency and to lack the stimulation associated with amphetamines. Further work will reveal if these agents have the selective profile and freedom from abuse liability and other serious side effects that are required in an advanced therapeutic agent.

A group of long-acting o-aminoalkylbenzhydrols (IV) have been reported that have moderate anorexic potency with little stimulation, whose pharmacological profile and structure-activity relationships suggest a mechanism of action different from amphetamine.[37,38] Structural resemblance to a more potent compound, mazindol (III), may be seen in this group.

A series of aryloxyalkylamines with cyano and sydnone functional groups on the aryl moiety (V) have been prepared and tested.[39] This series produced some potent anorectics which also antagonized reserpine hypothermia. Certain of the active agents caused severe stereo-typical behavior in cats at moderate to high doses.



IV



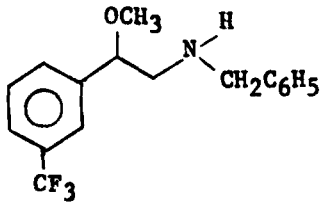
V

	X
a	4-Cl (Cpd. F-36)
b	4-Cl, 3-CF_3
c	3-CF_3

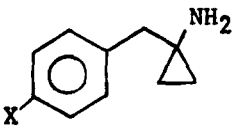
X = sydnonyl, cyano
R' - C_2H_5,n-C_4H_9,-$(CH_2)_4$
R = H, CH_3

The pharmacology[16], structure-activity relationships[40] and metabolism[41] of a new anorectic agent, a beta-methoxyphenethylamine bearing an aryl-CF_3 group were reported. The profile of the lead compound, SK&F 1-39728 (VI), resembled that of fenfluramine showing potent anorexia with mild sedation. The compound was relatively non-toxic and was resistant to tolerance development. Brain biochemistry has been investigated.[30]

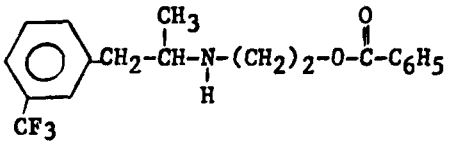
An extended series of 1-benzylcyclopropylamines (VII) were prepared and evaluated as anorectic agents.[42] The 4-chloro compound showed the highest potency in both rat and dog anorexia tests followed by the parent of the series and the 4-CH_3 analog. The 3-CF_3 analog was only weakly active.



VI SK&F 1-39728



VII X=Cl,CH_3,H

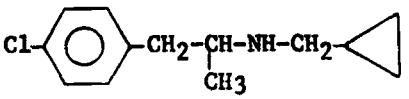


VIII S992

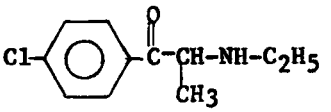
The fenfluramine derivative S992 (VIII) was described as a potent anorectic with a high therapeutic ratio and lack of untoward effects in animals.[13b] In man, it produced a significant loss in body weight[43,44] with increases in plasma triglycerides and ketones[44] and a decrease in serum cholesterol concentration.[44] Side effects included dry mouth, depression, irritability, and reduction in cigarette and alcohol consumption.[44]

Compounds related structurally to amphetamine (containing the carbon skeleton of the parent) were further investigated with some progress in improving anorexiant selectivity. Clinical study of a p-chloroamphetamine analog, A-31960 (IX)[45] showed modest potency for the drug but low stimulation liability and minimal cardiovascular effects compared to methamphetamine and diethylpropion controls.

A compound (X) related to diethylpropion was found to be an effective appetite inhibitor in clinical trial.[46] Improved control of side effects by use of sustained release medication was shown.



IX A-31960



X SK&F 70948

Further reports appeared on N-substituted amphetamines, several based on clinical trials. Most investigators recognize that these compounds function perhaps entirely by release of amphetamine itself by metabolic dealkylation[47] or deacylation (see metabolism section). Thus their profile is basically that of amphetamine attenuated with respect to anorectic potency, stimulation and side effects but with prolonged duration of action.

Structure-Activity and Side Effects - Structure-activity relationships received first consideration in the recent Symposium on Amphetamines.[13a-d,q,x,ad] Anorexic phenethylamines have been ranked and grouped according to their anorexic potency and their activity in a continuous avoidance behavior test.[15] Fenfluramine produced potent anorexia and decreased avoidance responding. Most compounds caused increases in avoidance, methamphetamine and p-chloroamphetamine being most potent. Para-methylamphetamine was potent at causing decrease in avoidance.

Pulmonary hypertension has been found to be a possible side effect of anorexic agents, notably aminorex,[48] Attempts to show a pathogenetic mechanism have been inconclusive.[49] The effect seems to be localized in pulmonary small blood vessels. A study of chlorphentermine in rats showed development of abnormal cells in the alveoli.[50]

Mechanisms of Anorectic Action - Amphetamine, fenfluramine and, to a lesser extent, other anorectics have been studied intensively to relate the behavioral effects of these drugs to biochemical parameters in the brain. The large sections in the Milan Symposium Book[13j-v] give an accurate description of the state of the art as of 1969. Despite this intense interest, agreement has not been reached on amphetamine's mechanism of stimulant or anorexiant action.

A simplified summary of recent studies on amphetamine actions and drug interactions follows:

Amphetamine: Releases and depletes brain norepinephrine.[13h,j,s,u,v]
Increases brain dopamine turnover.[13l]
Releases serotonin (higher doses).[13m]
Releases its own stored metabolite, p-hydroxynorephedrine, a false transmitter.[13j,k]

Amphetamine anorexia is inhibited by:

Tyrosine hydroxylase inhibitors.[51,54]
Reserpine pretreatment (norepinephrine, serotonin releaser.[41,52,53]
p-Chlorophenylalanine pretreatment (a serotonin depletor)[55,56] (stimulation also blocked).
Cyproheptadine pretreatment (serotonin antagonist).[56]

Amphetamine anorexia is enhanced by desmethylimipramine, a blocker of norepinephrine re-uptake, but is unaffected by chlorimipramine, a blocker of serotonin re-uptake.[57,58]

The above observations have been used with a great deal of additional data to reach the conclusion that amphetamine exerts its anorectic action principally through catecholaminergic mechanisms and is mediated by norepinephrine and dopamine release.[13m,59] Serotonergic mechanisms are also

involved but play a secondary role in the case of this drug.[56] However, release of serotonin by fenfluramine or norfenfluramine may be of primary importance to the anorectic effects of these two drugs.[60,58] Other phenethylamine-based anorectics have been studied less but appear to display varying profiles.[56,57,61] Some share certain effects with amphetamine, others have effects in common with norfenfluramine or fenfluramine. To a certain extent each agent seems to have its own unique profile.[62]

Alpha and Beta Hypothalamic Receptors - A unifying hypothesis of hunger and satiety systems in the lateral and ventromedial hypothalamus has been proposed.[63] The lateral hypothalamus contains beta receptors; beta agonists stimulate these receptors to produce the behavioral response of satiety. Beta antagonists block satiety. The ventromedial hypothalamus contains alpha receptors; stimulation by alpha agonists produces the behavioral response of hunger. Alpha antagonists block hunger. The perifornical area contains both types of receptors and elicits attenuated responses. This revolutionary hypothesis has thus far brought little published response. Results in sheep and cattle tend to partially support and partially contradict these conclusions.[64]

Tolerance and Withdrawal - A clinical study of withdrawal following prolonged amphetamine abuse showed temporal correlation of excretion of a norepinephrine metabolite, 3-methoxy-4-hydroxyphenyl glycol, with depression.[65]

Two studies of tolerance to the anorexic effects of amphetamine brought out that this tolerance reflects a behavioral adaptation of the animal to the drug. Animals dosed with amphetamine following feeding responded normally to the drug when, after several days, the drug dose was given before the meal.[66,67]

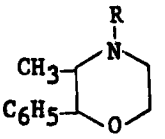
Lipolysis - Amphetamine-induced lipolysis may be mediated via release of a pituitary hormone or by an action on the hypothalamus.[68] Release of catecholamines from the adrenals as a mechanism was eliminated by adrenalectomy. Hypophysectomy, thyroidectomy or cortisone pretreatment inhibited the lipolytic effect. This effect was also blocked by propranolol. A tolerance study indicated rapid development of tolerance to both anorexia and mobilization of fatty acids.[69] The animals remained responsive to the administration of exogenous norepinephrine but developed cross-tolerance to the lipolytic effect of other anorectics.

Metabolism - The understanding of the metabolism of anorectic drugs continues to grow in importance.[13e-h] Interaction of parent drug with principal metabolites has been shown to play an important role in overall drug profile.[13j,k]

Dealkylation and deacylation of N-substituted phenethylamine anorexics are likely to be rapid metabolic processes.[41,13d,f] Aromatic hydroxylation is greatly dependent on aromatic substitution[13d,e]; beta hydrox-

ylation is to a certain extent an unknown quantity.[13f] Oxidative deamination of the side chain ultimately leading to the corresponding benzoic acid is an important metabolic process of virtually every member of this group of drugs.[13f,41]

N-Hydroxylation has been recently reported[70] to be an important metabolic transformation in the case of phendimetrazine (XI):



XI

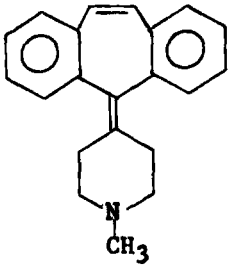
a, R = CH_3, phendimetrazine
b, R = OH, metabolite

Miscellaneous - Biguanides continue to receive some attention for the treatment of obesity. A clinical study has demonstrated weight loss in excess of that caused by the degree of anorexia produced in five obese women treated with phenformin.[71] There was no protein catabolism, change in metabolic rate or water loss involved. In addition to inducing anorexia, biguanides have been suspected of interfering with absorption.[72] A study in gold thioglucose treated mice demonstrated body weight loss without anorexia with phenformin[73] implicating factors other than anorexia as the cause of phenformin's antiobesity effect.

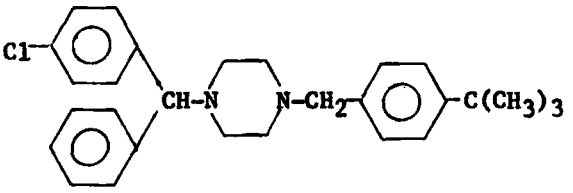
Further studies were conducted on the fat mobilizing substance FMS 1A, a glycopeptide isolated from urine that causes anorexia in animals and man. A clinical study in man has demonstrated that exhaustive physical exercise produces an increase in the plasma concentration of FMS 1A.[74] Studies in rats indicated that high protein diets caused an increase in the amounts and total anorexigenic activity of FMS 1A.[75] The amounts and total activity of FMS 1A excreted in the urine were found to be directly proportional to the amounts of food consumed.

Prostaglandins have aroused some interest in obesity research.[76,77] Subcutaneous and hypothalamic injections of prostaglandins to male rats decreased food intake compared to controls.[76] They have also been shown to affect lipolysis, growth hormone levels, glucose uptake by adipose tissue and synthesis of triglycerides from glucose and acetate and have been suggested as having a physiological role as mediators for the control of free fatty acid mobilization.[77] A study of the effect of diethylstilbestrol on food intake of mice with ventromedial hypothalamic lesions reported that these animals are supersensitive to the anorexigenic activity of diethylstilbestrol[78] just as other hypothalamically lesioned animals are hypersensitive to the effects of anorectics.[79]

New test methods in animals for detecting anorectic and antiobesity drugs have received little attention in the past few years. One test method in cats was described utilizing injection of sodium pentobarbital into the cerebral ventricles producing hyperphagia which can be inhibited by prior administration of anorectic drugs.[80] Another test method in high fat diet rats was shown to be capable of detecting drugs that affect body weight with or without reducing food consumption.[34]



XII cyproheptadine



XIII buclizine

Appetite Stimulants - The field of appetite stimulants represents a ripe area for research. The number of drugs shown to be useful and safe for this indication is limited and a significant new agent could command a major part of a fairly sizeable potential market. The drug most used for stimulation of appetite is cyproheptadine (XII), an antihistaminic serotonin antagonist. Reports in the past three years have shown that cyproheptadine was effective in stimulating appetite and promoting weight gain in basically normal underweight children[81,82], in children with any psychological problems[83], in underweight but healthy adults[84] and in anorexic patients[85] but was not effective in healthy but underweight older psychiatric patients.[86] It is thought that the weight gain is principally due to an increase in food intake without an effect on fasting blood glucose or serum insulin levels.[87] Buclizine (XIII), an agent with tranquilizer and antihistaminic activity, was also shown to be effective in producing appetite stimulation and increasing body weight in children.[88]

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Section II - Pharmacodynamic Agents

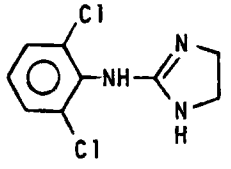
Editor: John G. Topliss, Schering Corp., Bloomfield, New Jersey

Chapter 7. Antihypertensive Agents

Anthony M. Roe, Smith Kline and French Research Institute,
Welwyn Garden City, Hertfordshire, England.

Introduction - Between the years 1967 and 1971, 2,725 people were found to be hypertensive (blood pressure > 160/95) out of a group of 22,929 people who were screened.[1] The magnitude of the problem presented by this "controllable disease"[2] is manifest and urgent, because the advantages of early treatment are becoming generally recognised.[3,4] A symposium[5-8] on "Hypertension: Mechanisms and Management" describes the present status of the disease; other reviews[9-11] discuss the chemotherapeutic control of high blood pressure, and the mechanism of action of some vasoactive drugs.[12] The interaction of antihypertensive agents with other drugs has been summarised on a logical mechanistic basis.[13] The spontaneously hypertensive rat (SHR) has been further assessed[14-17] as a model for human essential hypertension.

In a review on hypertension and the geochemical environment, the evidence that cadmium may be involved in the genesis of essential hypertension is discussed and has led to the intriguing speculation of a link between cadmium and the well-known epidemiological correlation of cardiovascular disease with the softness of the water supply.[18]



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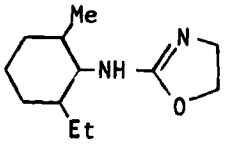

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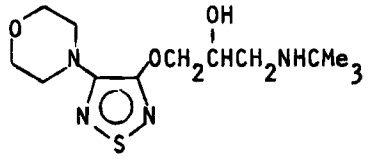


III

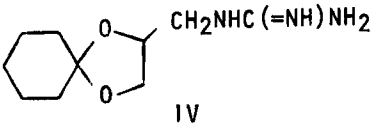
The clinical study of recent antihypertensives - Many papers continue to demonstrate the therapeutic value of clonidine (I).[19] Its detailed mechanisms of action continue to be explored in man and animals.[20-22] Its centrally mediated effect on the sympathetic system has been shown in man,[23] and it has been concluded from studies on isolated human tissue that non-competitive blockade of noradrenaline in venous tissue may also be responsible for its hypotensive action.[24] Other work has shown that, at very low concentrations, clonidine and BAY a 6781 (II) inhibit the release of transmitter in postganglionic adrenergic nerves of rabbit heart and postganglionic cholinergic nerves of guinea pig ileum.[25,26] The reduction of the hypotensive response in rabbits produced by clonidine after treatment

with desmethylimipramine is also interpreted as clonidine acting on noradrenergic neurones rather than at the α-adrenergic receptor.[27]

The efficacy of β-adrenoreceptor antagonists such as propranolol,[28-31] alprenolol,[28] oxprenolol,[32] pindolol,[33] practolol,[34] and MK-950 (III)[35,36] in many patients is well documented, although the mechanism by which they act is not understood. A possible resolution of this "therapeutic paradox"[37] has been proposed[38] following the important observation that patients with high plasma renin activity show the greatest falls in both renin level and blood pressure after propranolol, whereas this drug does not reduce the blood pressure or the renin level in patients with low plasma renin levels. The therapeutic utility of combining propranolol (and other β-adrenoreceptor antagonists) with a vasodilator[39-45] is probably due to propranolol's ability to inhibit renin secretion in addition to its effect on the associated tachycardia.

The use of vasodilators alone is exemplified by the efficacy of minoxidil[46] and guancydine.[47] Further clinical studies with minoxidil have shown that the prolonged antihypertensive response is not due to slow excretion or metabolism, but may be due to a persistent effect on receptor sites.[48] Indoramine is an effective α-adrenoreceptor antagonist in man[49] and it appears to be well tolerated.[50]

The correlation of the clinical effects of guanethidine and debrisoquin with information obtained from in vitro studies has been reviewed.[51,52] Guanadrel (IV, U-28,288D)[53,54] appears to act more rapidly than guanethidine and to be equally effective. The novel β-arylserine (V, Ro 4-2137)[55] only exhibits slight antihypertensive activity in man.



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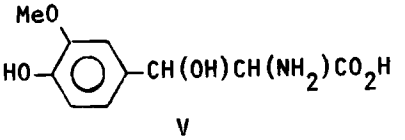

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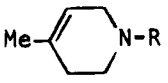

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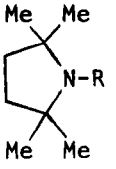

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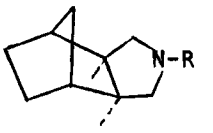
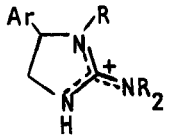


VIII

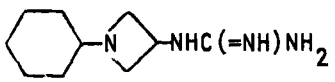
R = $CH_2CH_2NHC(=NH)NH_2$

Adrenergic neurone blockers - Structure-activity relationships of an extensive series of N-guanidinoethyl-azacycloalkanes have been discussed;[56] the compounds ranged from the clinically active cyclazenine (VI) to the pure ganglion blockers (VII) and (VIII). Potent adrenergic neurone blocking

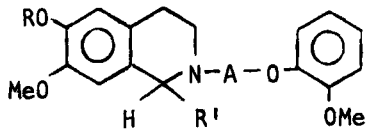
activity has been reported for compounds such as (IX).[57] AZ-55 (X)[58] affects the responses to stimulation of rabbit atria and aortic strips in a similar manner to that of guanethidine. The tetrahydroisoquinolines (XIa and b) are α-adrenoreceptor antagonists, and this action is combined with adrenergic neurone blockade in (XIc, SC 3123)[59] which causes hypotension in dogs, cats and rats,[60] but proved unsatisfactory in the clinic.[59]



IX



X



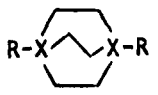
XI

a) R = R' = Me
A = CH_2CH_2

b) R = H, R' = Me
A = $C(=NH)NH(CH_2)_2$

c) R = Me, R' = H
A = $C(=NH)NH(CH_2)_2$

Ganglion blockers - The relationship between inter-nitrogen distance and ganglion blocking potency of bis-quaternary salts has been studied by several groups. Compound (XIIc), in which the inter-nitrogen distance is 6.5-7.7Å, is more active than (XIIa), (XIIb) or hexamethonium; it is hypotensive in the anesthetized rat at 1.0 mg/kg.[61] Compound (XIII) is also more active than hexamethonium.[62] Lipophilicity of these and other bis-quaternary salts is not a crucial factor in determining ganglion blocking potency.[62] Some bis-quaternary ammonium derivatives of cholane, in which the inter-nitrogen distance is about 5.8Å, required 7 mg/kg (i.v.) to produce the same effect on the blood pressure of the anesthetized cat as 2 mg/kg of hexamethonium.[63]

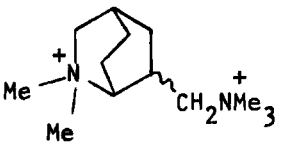


XII

a) X-R = $\overset{+}{N}Me$

b) X-R = $\overset{+}{C}NMe_3$

c) X-R = $CCH_2\overset{+}{N}Me_3$



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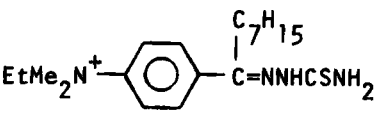
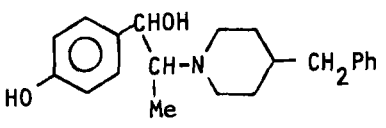


XIV

Hypotension, which may be a result of ganglion blockade, has been observed in the cat, dog and chick after the neuromuscular blocking agent M&B 15,944 (XIV).[64]

α-Adrenoreceptor blockers - The 4-hydroxyphenylpropanolamine (XV, RC 61-91, Vadilex) causes dose-related hypotension in rats, rabbits and dogs; it is two to five times more active than isoxsuprine as a vasodilator.[65] At 1.0 to 10 mg/kg, compound (XVI), and related arylpiperazines, lower the blood pressure of anesthetized dogs by 15-40%.[66] Rather specific and fairly prolonged α-adrenoreceptor blockade occurs in dogs treated with WR-149,024 (XVII).[67]

Further details have appeared on the properties of (XVIII): in several species it was a more potent hypotensive than its substituted derivatives which, although structurally related to clonidine, act mainly by peripheral α-adrenoreceptor blockade.[68]



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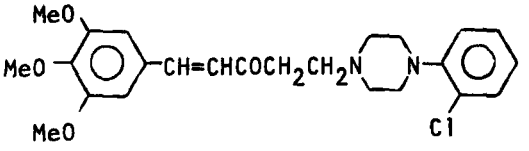

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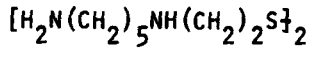
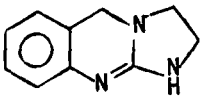


XVII



XVIII

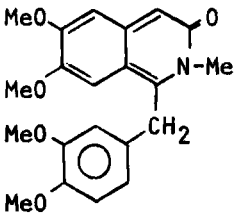
Compounds affecting adrenergic transmitters - The hypotensive effect of oral 5-(3,4-dibromobutyl)picolinic acid in normotensive rabbits and SHR's may be more persistent than that of 5-butylpicolinic (fusaric) acid itself,[69] which was antihypertensive in clinical trials.[70] In renal hypertensive rats, oral 3-amino-2-oxazolidinone and several of its Schiff's base derivatives lower blood pressure more than pargyline; their in vivo monoamine oxidase inhibitory activity does not correlate with antihypertensive potency.[71]

Several papers show that stimulation of central α-adrenoreceptors, which results in the inhibition of sympathetic tone, is the principle mode of action of α-methyldopa;[72-75] a similar mechanism has been demonstrated for l-dopa by dog cross-circulation experiments.[76,77] The potential clinical applications of dopamine analogs in cardiovascular disease have been reviewed.[78]

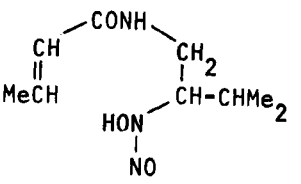
Naturally occurring and related compounds - The vasodilatation of twenty four natural and synthetic prostaglandins has been compared in the anesthetized dog, but none was more active than PGE_1.[79] The depressor effects of intravenous PGA_2 and PGE_2 in the SHR are thought to be mediated by a central effect on the vagus;[80] PGA_2 may be involved in the regulation of blood pressure and renal homeostasis.[81] Prostaglandin deficiency may play a role in the genesis of essential hypertension;[82] a prostaglandin-like substance which depresses adrenergic neurone activity is liberated when the perfusion pressure of the kidney is reduced.[83]

A heptapeptide amide and some derivatives having the 5-11 sequence of eledoisin show eledoisin-like activity in the dog.[84] [Sar^1,Ile^8]angiotensin II[85,86] is a long-lasting competitive inhibitor of angiotensin II. Neotetrazolium reduces the pressor effects of angiotensin by a complex mechanism.[87] A synthetic nonapeptide, pyroGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro (BPP_9a, SQ 20,881),[88] originally isolated from the venom of Bothrops jararaca, is a powerful competitive inhibitor of the enzyme which converts angiotensin I into angiotensin II, and may have utility in some forms of hypertension.[89] The angiotensin I analogs of previously described angiotensin II antagonists have now been prepared[90] and shown to inhibit the effects of both angiotensin I and II in the rat and in isolated tissue.

Methyl reserpate has been esterified with various cinnamic acids, and the products were given intravenously to conscious cannulated SHR's; the ester derived from 3,4-dimethoxycinnamic acid has a similar activity to rescinnamine.[91] Two recently characterized ergot alkaloids of the chanoclavine-type, rugulovasine A and B, elicit various responses including hypotension in rats and cats, the mechanism is thought to be depression of central vasomotor and accelerator centres.[92,93] In dogs, the 3(2H)-isoquinolone (XIX) causes more prolonged vasodilatation than papaverine but less positive inotropic effects.[94] A dopamine-β-hydroxylase inhibitor, dopastin (XX), has been isolated from a bacterial culture and synthesized;[95] the blood pressure of SHR's is lowered by 40-50 mm Hg after dopastin at 20 mg/kg (i.p.).[96]



XIX

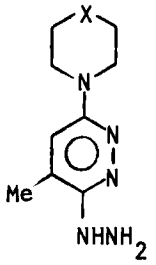


XX

Other hypotensives - The antihypertensive activity of an extensive series of diazoxide analogs has been subjected to multiple regression analyses using lipophilic, electronic and steric substituent constants.[97] This is an impressive example of the application of these methods, and leads to a remarkably simple definition of the substituent requirements for maximum activity in this series and to some conclusions about possible receptor site interactions.

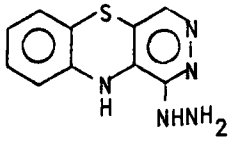
The minimum effective dose of monosubstituted sulfamoylazides needed to lower the blood pressure of anesthetized normotensive dogs is 0.005-0.01 mg/kg (i.v.), and of the disubstituted derivatives it is 0.3-1.25 mg/kg. This striking difference is related to the extreme stability to hydrolysis of the latter, azide ion being the hypotensive species.[98]

Compounds (XXI) appear to be the most potent of some new hydrazinopyridazines; they lower the blood pressure of Grollman rats at 3 mg/kg (p.o.) and anesthetized dogs at 0.5 mg/kg (i.v.).[99] At 6.25 mg/kg (p.o.) the hydrazine (XXII) lowers the blood pressure of conscious normotensive rats by 13-50% for several hours.[100]

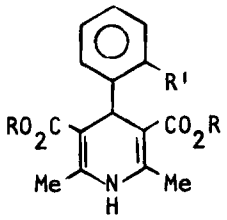


XXI

(X = CH_2 or O)



XXII



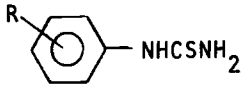
XXIII

a) R = Et, R' = CF_3

b) R = Me, R' = NO_2

SK&F 24260 (XXIIIa) is the most interesting of a series of potent direct-acting vasodilators.[101] Sustained hypotension is observed in anesthetized dogs at 0.01 mg/kg (i.v.), and in normotensive, neurogenic or renal hypertensive dogs at 1 mg/kg (p.o.) accompanied by tachycardia. SK&F 24260 is also active in other species, including hypertensive humans at 0.1 mg/kg (p.o.).[102] Nifedipine (XXIIIb, BAY a 1040) which has been developed as an anti-anginal agent,[103] is an orally active coronary dilator which also has significant hypotensive activity at a single oral dose of 20 mg.[104]

Several arylthioureas (XXIV a-d) are antihypertensive in the metacorticoid rat at 1 mg/kg or less (p.o.), but they are not active in the neurogenic dog. A metabolite (XXIVe) from both the rat and the dog was itself active only in the dog; the S-dioxide of (XXIVb) is orally active in both species.[105]



XXIV

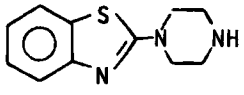
a) R = 2,6-Cl_2

b) R = 2,6-Me_2

c) R = 2,6-$(MeO)_2$

d) R = 2-Me

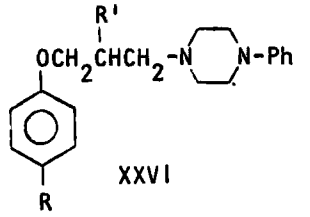
e) R = 2,6-Cl_2-4-HO



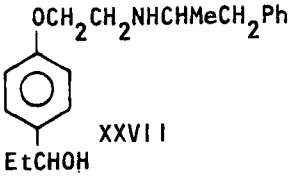
XXV

The benzothiazole (XXV) is antihypertensive in rats, having an ED_{50} of 6 mg/kg (s.c.).[106] Oral 2-acetamidothiazole and some related 1,3,4-thiadiazoles cause prolonged reduction of the blood pressure of conscious rats, but tolerance is rapidly established.[107]

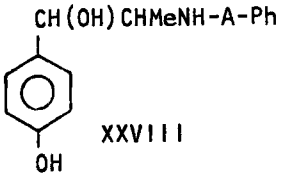
The blood pressure of anesthetized cats is lowered by 50-80 mm Hg for longer than one hour by 2.5 mg/kg of the piperazines (XXVI a-d).[108] The cardiac stimulant (XXVII) is a potent peripheral vasodilator; after 2 mg/kg (i.v.), the mean arterial pressure of the dog anesthetized with chloralose falls by about 40 mm Hg for more than an hour.[109] Compounds (XXVIIIa and b) show papaverine-like activity in the rabbit; (XXVIIIa) lowers the blood pressure at 10 µg/kg (i.v.).[110]



XXVI



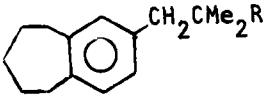
XXVII



XXVIII

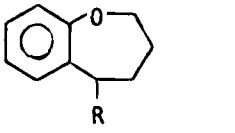
a) R = EtSO, R' = OH
b) R = PhCH=CHCO, R' = OH
c) R = $PhCH_2CH_2CO$, R' = OH
d) R = EtCO, R' = H

a) A = $(CH_2)_3S$
b) A = $CHMeCH_2S$

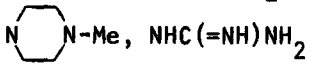


R = NH_2, NHMe

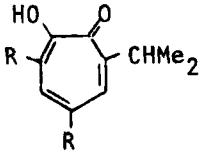
XXIX



R = $NHMe$, NMe_2,



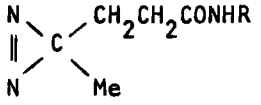
XXX



R = CH_2- 

XXXI

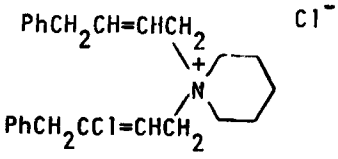
The amines (XXIX) cause a protracted fall in the blood pressure of DOCA rats at 8-10 mg/kg (i.v.).[111] Several tetrahydro-1-benzoxepins (XXX) cause hypotension at 5-30 mg/kg (i.v.) in rats.[112] The tropolone (XXXI) is more potent than papaverine as a peripheral, and especially as a coronary, dilator; its hypotensive effect may also be due to vasomotor inhibition.[113]



XXXII

R = CH_2Ph, 

XXXIII



In a study of the pharmacological actions of miscellaneous diazirines, (XXXII) turned out to lower the mean blood pressure of conscious normotensive rats by 20% or more two hours after dosing at 100 mg/kg (p.o.).[114] The quaternary salt (XXXIII) is the most active of several analogs which cause sustained hypotension in cats by an unknown mechanism.[115] At 100 mg/kg, parenteral 2-mercaptopropionylglycine (Thiola) lowers the blood pressure of anesthetized rats by 30-55 mm Hg for 4-6 hours; vascular or capillary dilation is the suggested mechanism.[116]

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CHAPTER 8. ANTIARRHYTHMIC AND ANTIANGINAL AGENTS

Gilbert W. Adelstein and Richard R. Dean,
Searle Laboratories, Chicago, Ill.

ANTIARRHYTHMIC AGENTS

Introduction - A cardiac arrhythmia is caused by an abnormality in the rate, regularity or site of origin of cardiac impulses, or by disturbances which affect the sequence of activation of the atria and ventricles. Thus arrhythmias may be said to result from abnormalities of impulse formation (automaticity), impulse conduction, or both.[1-5]

It is possible to identify useful properties of antiarrhythmic drugs on the basis of their effects on automaticity and conduction. Most of the known antiarrhythmic drugs reduce ectopic activity due to automaticity. Antiarrhythmic compounds effective against impaired conduction arrhythmias, however, can be categorized as two major types. The first type represented by quinidine, procaine amide, and propranolol shifts the membrane responsiveness curve to the right; that is, it decreases dv/dt for any given resting potential. The result is that an impulse traveling through depressed tissue may be completely blocked thereby precluding reentry.[6]

The second type of drugs represented by lidocaine and diphenylhydantoin shifts the membrane responsiveness curve to the left. This results in improved conduction in a previously depressed pathway so that reentry is minimized.[6]

The common feature of both types of drugs is the fact that the effective refractory period is generally prolonged to a greater degree than the action potential duration. This has been postulated as a major mechanism in the effectiveness of these drugs.[7]

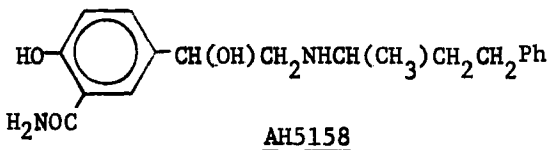
Vaughn-Williams[8] separated all antiarrhythmic drugs into three main classes of action: (1) those which directly interfere with depolarization (local anesthetic type), (2) those with an anti-sympathetic action by direct neurone blockade or transmitter competition (β-blocker) and (3) those which delay repolarization. Compounds can be placed in one or more classes. Thus, quinidine acts via classes 1 and 3 actions, amiodarone via classes 2 and 3, bretylium only via class 2, etc.

Recent studies on verapamil led Vaughan-Williams to suggest a fourth mechanism of action; namely, that of blocking a calcium-carrying depolarizing current.[9,10] While Vaughn-Williams' classification is useful in categorizing some of the diverse, indirect, mechanisms of action, the previously described theory[6,9] of arrhythmia genesis and drug classification provides a better insight into some of the common functional end-points which result from these varied mechanisms.

Beta-Receptor Blocking Agents - Although the synthesis and evaluation of compounds as β-receptor blockers continues at a strong pace, more recent attention has turned to the separation of antiarrhythmic and β-blockade features of the aryloxypropanolamine moiety. The separation of these features became apparent when it was demonstrated that d-propranolol and dl-propranolol were equipotent against ouabain-induced arrhythmias, while the dextro-isomer of propranolol has only 1/60-1/10 the potency of the racemic mixture as a β-blocker.[11,12] It has been suggested that the administration of β-receptor blocking drugs may lead to deterioration of cardiac function[13,14] in patients with congestive heart failure in whom the circulation is supported by sympathetic activity.[15,16] Accordingly, it was considered that if the clinically effective antiarrhythmic activity is not dependent upon the adrenergic blocking properties of these drugs, their potency as β-receptor blocking agents might actually be undesirable.

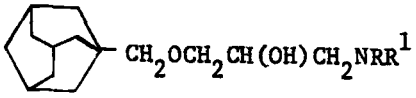
The antiarrhythmic effects of several β-blockers have been compared to each other with regard to potency and depressant properties.[17] For aconitine-induced atrial arrhythmias, the order of decreasing potency is: propranolol >MJ 1999, ajmaline >LB 46> quinidine. The antiarrhythmic effects of H56/28, ICI 50172, and DCI were not determined due to the cardiovascular depression observed in the dosage range used. For ouabain-induced ventricular arrhythmias, propranolol, LB 46, and H56/28 were similar, although heart rate and systolic blood pressure were significantly depressed with the latter compound. MJ 1999 and ICI 50172 were not effective in reversing ventricular arrhythmias.

AH 5158 was found to possess inhibiting activity at both α and β-receptors in a variety of in vitro tissue preparations.[18] The β-adrenoceptor blocking activity of AH 5158 was 5-18 times less than that of propranolol, while the α-adrenoceptor blocking activity of AH 5158 was 2-7 times less than that of phentolamine. In doses up to 1 mg/kg, AH 5158 caused a dose-dependent blockade of noradrenaline vasopressor responses in anesthetized dogs, but sympathetic blockade was "self-limiting" at doses beyond 1 mg/kg. Apparently higher doses of AH 5158 prevent re-uptake of noradrenaline allowing for increased levels of circulating catecholamines. At doses of 1-8 mg/kg, AH 5158 antagonized both catecholamine and ouabain-induced arrhythmias in anesthetized dogs, and at .25-5 mg/kg orally, systolic blood pressure was significantly decreased in conscious renal hypertensive dogs. A limited pilot study in humans showed that AH 5158 effectively lowered blood pressure.



AH5158

Adamantylmethoxypropanolamine I was less active than propranolol as a β-blocker.[19] Local anesthetic and antiarrhythmic properties were present but significantly less than with lidocaine or procaine amide.

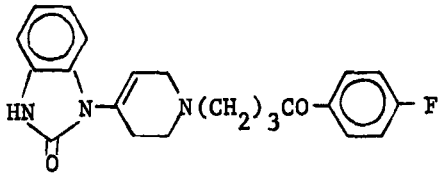


I, R = i-Pr, R^1 = H

The effects of LB-46 (pindolol; 1-(indol-4-yloxy)-3-(isopropylamino)-2-propanol) on coronary ligated dogs showed significantly increased maximum rate of rise of the first derivative of the left ventricular end-diastolic pressure, heart rate, and cardiac output.[20] These effects were more favorable than those produced by propranolol and comparable to those of bretylium. In isolated guinea pig atria stimulated by adrenaline, LB-46 was 8.3 times more potent than propranolol as a β-blocker, and five times more potent than propranolol as a β-blocker in the isolated chick rectum relaxed by adrenaline.[21] On the other hand, LB-46 was 20-25 times less potent than propranolol in preventing tachyarrhythmias in electrically stimulated isolated pig atria. LB-46 exerted a stimulatory effect in guinea pig atria which was prevented by reserpine pretreatment, indicating that the release of catecholamines from cardiac stores may be involved in the sympathomimetic effects of LB-46. A clinical study with LB-46 showed it to be effective in reversing ventricular arrhythmias, especially digitalis-induced arrhythmias.[22] It was less effective in converting atrial flutter and fibrillation to a sinus rhythm.

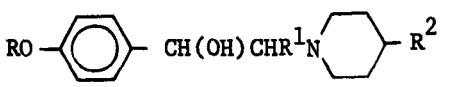
One of the most interesting drugs developed during the past two years is UM-272 (N,N-dimethyl-1-isopropylamino-3-{naphthyloxy}-propan-2-ol iodide). Although this compound is a dimethyl derivative of the β-adrenergic blocker, propranolol, it is strikingly different in terms of its pharmacological profile. UM-272 exhibits potent activity against digitalis and myocardial infarction arrhythmias in the dog, but unlike propranolol is neither a β-blocker nor a local anesthetic.[23,24] These great differences undoubtedly are due to the fact that UM-272 is a quaternary ammonium compound while propranolol is a secondary amine.[25]

Miscellaneous antiarrhythmic agents - Antiarrhythmic properties have been ascribed to several new series of compounds. Dehydrobenzperidol when administered to anesthetized dogs doubled the threshold dose of adrenaline or noradrenaline required to disrupt cardiac rhythm.[26] Because dehydrobenzperidol is a potent α-blocker, the antiarrhythmic effect may be due to hypotension mediated through the α adrenergic block.



Dehydrobenzperidol

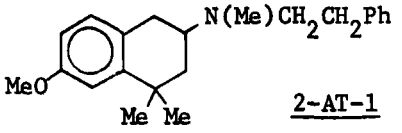
A series of 2-piperidinoalkanols related to phenethanolamines showed antiarrhythmic effects against aconitine in rats.[27] RC 61-91 was about



RC-61-91; R=H, R^1=Me, R^2=CH_2Ph

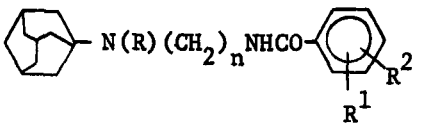
twice as active as quinidine, but this activity was accompanied by considerable α-receptor blockage and local anesthetic effects.

A series of 2-aminotetralins represented by 2-AT-1 showed antiarrhythmic activity in the mouse - $CHCl_3$ assay.[28,29] 2-AT-1 caused greater chronotropic depression than did either propranolol, quinidine, lidocaine or procaine amide at similar doses. However, 2-AT-1 caused less inotropic depression than with propranolol or lidocaine, but greater inotropic depression than with quinidine or higher doses of procaine amide. Increasing the lipophilicity of groups attached to the saturated ring tended to increase antiarrhythmic activity of 2-AT-1 but with a concomitant increase in toxicity.



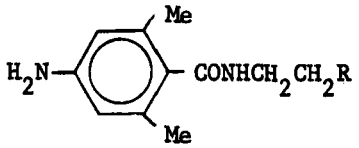
2-AT-1

Some adamantylaminoalkylbenzamide analogs of procaine amide were found active in the mouse-$CHCl_3$ assay.[30] II was three times as active as procaine amide in this test but 1.5 times as toxic, and this therapeutic ratio was maintained in dogs in an unspecified test.



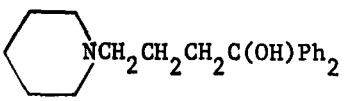
II; R=Et, n=2, R^1=H, R^2=p-NH_2

In a series of dimethylbenzamide analogs III in which the side chain nitrogen is cyclized, antiarrhythmic effects against ouabain and aconitine in anesthetized cats were qualitatively similar to procaine amide.[31]



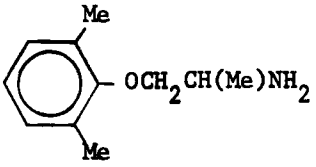
III, R=N

Diphenidol, an anti-emetic agent, has been reported to effectively protect dogs against digoxin intoxication.[32] At higher doses, diphenidol had no effect on cardiac output or total peripheral vascular resistance. It was suggested that diphenidol might find clinical use in the treatment of digitalis intoxication because of the drug's effect of shortening A-H conduction time and depressing enhanced ventricular automaticity induced by digitalis intoxication.



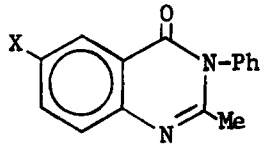
Diphenidol

An anti-convulsant agent, Kö 1173, was reported to be effective in reversing arrhythmias induced by adrenaline, ouabain, and coronary ligation in dogs.[33] Kö 1173 was found to be at least as effective as phenytoin in abolishing ventricular ectopic beats in conscious dogs after coronary ligation.



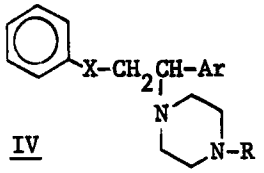
Kö 1173

In a series of quinazolones related to methaqualone, QZH-6 and QZH-7 were found to be more potent than quinidine in suppressing aconitine-induced atrial arrhythmias, and coronary ligation and adrenaline-induced ventricular arrhythmias in dogs.[34] Both QZH-6 and QZH-7 were found to be seven times less toxic to the myocardium than quinidine on a weight to weight basis, as judged from their effect on the competence index in the heart-lung preparation of the dog.



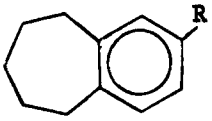
QZH-6, X = Br
QZH-7, X = I

A series of diarylpropylpiperazines IV were active against aconitine and chloroform-induced arrhythmias in the rat.[35] Phenethylamines V fused through the aromatic ring to cycloheptane were reported as effective in



IV

X = CH_2
Ar = Ph
R = Me

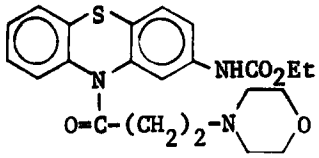


V

R=$CH_2CH_2NH_2$

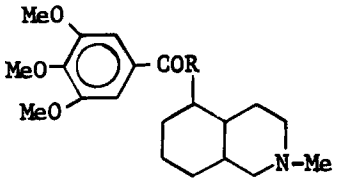
preventing chloroform-induced fibrillation in the mouse, but no data was given.[36]

Antiarrhythmic activity has been ascribed to ethyl 10-(β-morpholinopropionyl) phenothiazine-2-carbamate (ethmosine)[37] which had 33 times the activity of quinidine on the isolated rabbit atrium.



Ethmosine

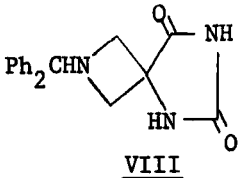
A series of decahydroisoquinolines VI and VII were examined with respect to their distribution coefficients and acid dissociation constants.[38] The trans-isomers were more lipophilic than the corresponding cis isomers, and the pKa



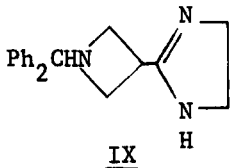
VI, R = O
VII, R = NH

of each isomer was not significantly different from the other. On this basis, it was suggested that the greater activity of the trans-isomer was due in part to its increased binding to the cardiac cell membrane.

Antiarrhythmic activity was ascribed to the spirohydantoin VIII and imidazoline IX both containing an azetidine ring.[39] Although both VIII and IX were effective against $CHCl_3$-induced fibrillation in the mouse, only IX was effective against arrhythmias induced by coronary ligation in the dog. In addition, IX was cardiodepressant in cats.



VIII



IX

ANTIANGINAL AGENTS

Angina pectoris results from an imbalance between the oxygen supply and the oxygen demand of the myocardium. Oxygen delivery may be inadequate because of diminished caliber of the coronary vessels, decreased perfusion pressure, arterial hypoxia, decreased oxygen carrying capacity of the blood, increased affinity of hemoglobin for oxygen or decreased blood flow due to high blood viscosity. Myocardial oxygen demand is increased by increased heart rate, myocardial wall tension or inotropic agents.[40] In man angina pectoris is often preceded by a rise in heart rate, systemic arterial pressure, and, therefore, increased oxygen needs.[41,42]

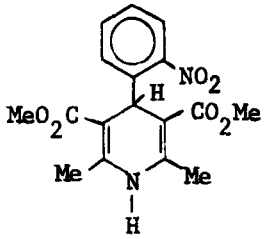
Therapy has been aimed at reducing ischemia by increasing the oxygen supply to the heart or decreasing oxygen needs as mentioned in recent reviews.[43-49]

Organic Nitrates - Recent studies in animals and man have shown that nitroglycerin and pentaerythritol tetranitrate redistribute coronary blood flow to ischemic areas without necessarily increasing total blood flow. This mechanism is unique compared to vasodilators such as dipyridamole, chromonar, lidoflazine, iproveratril, papaverine and prenylamine[50] and results from direct dilation of coronary collaterals. Other studies have shown that nitrates reduce peripheral resistance and blood pressure and by decreasing central venous pressure reduce myocardial oxygen needs.

Nitroglycerin remains the drug of choice for relief of ischemic heart disease in spite of its short duration of action. Research efforts directed towards improving the efficacy of organic nitrates via chemical modification have led to the so-called long-acting nitrates, such as isosorbide dinitrate (ISD) and pentaerythritol tetranitrate (PETN). These latter agents, available orally, have a somewhat delayed onset of action, but are still effective long after sublingually administered nitroglycerin is rendered inactive. Another approach involves the use of nitroglycerin itself in some dosage form, such as time-release granules, to extend its

biological lifetime. Needleman and co-workers[51] have criticized the rationale for use of long-acting organic nitrates by indicating that the liver rapidly clears organic nitrates from the general circulation and that the partially de-nitrated metabolites retain only a very low order of antianginal activity. Several reviews have appeared describing the use of nitroglycerin[52] and organic nitrates[53] in the treatment of angina pectoris.[54]

A clinical study has shown that BAY a 1040 (nifedipine), a nitrophenyldihydropyridine derivative, was effective in relieving anginal attacks in a variety of patients.[55] Bay a 1040 tended to lower blood pressure in most patients and considerable improvement in the T wave of abnormal ECG's was observed.



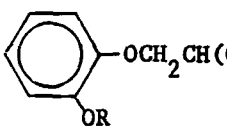
BAY a 1040

Beta-Receptor Blocking Agents - The β-blocker, propranolol appears to have gained widespread use in the treatment of angina pectoris although it has not been approved by the FDA for this use.[43,56,57] The beneficial effect of propranolol appears to be primarily due to a decrease in oxygen demand but this may not be entirely due to β-adrenergic receptor blockade since high doses are often needed.[58]

β-blockers may be acting through two additional mechanisms. Propranolol, like nitroglycerin has been shown to redistribute blood flow into ischemic regions of the heart.[59-62] Since it is presumed that resistance arterioles in the ischemic area are fully dilated, an unmasking of α-receptor constrictor activity in normal regions by β-blockade would result in a shunting of blood to the more dilated ischemic arterioles.

A very recent study indicates that propranolol affects the hemoglobin dissociation curve (HDC) resulting in an increase in the P_{50} or the partial pressure of oxygen at which hemoglobin is 50% saturated.[63] Thus, propranolol, but not nitroglycerin, decreases the affinity of hemoglobin for oxygen thereby releasing more oxygen within cells where pO_2 is reduced. Although the contribution of this mechanism to propranolol's overall effect is not known, the theoretical implications of such an action have been discussed by Guy.[64]

Several new series of β-receptor blocking agents and their effects on test animals have been reported. A high degree of β-blocking activity was ascribed to a series of o-alkyloxy- and o-aralkyloxyphenoxypropanolamines.[65]



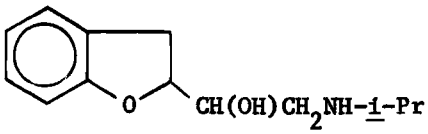
X

R= 2-furfuryl
R^1= t-butyl

The 2-furfuryloxy derivative X had a particularly high pA_2 value of 9.05 as determined by antagonism to isoproterenol in an

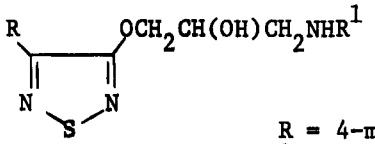
isolated strip of guinea pig trachea.

Replacement of the naphthalene ring of propranolol by quinoline, benzofuran, benzothiophene, indole, methylenedioxyphenyl, or oxoxanthene did not appreciably alter their activity as β-blockers.[66] Likewise, replacement of the naphthalene ring of pronethalol by indole, quinoline, phenothiazine, benzodioxan, chroman, dibenzofuran, or quinazoline caused little change in activity, whereas compounds such as XI that incorporate features of the propranolol type showed considerable β-blocking activity.[67]



XI

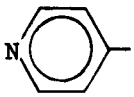
Long acting β-blockers were prepared by substitution of the aromatic nucleus of propranolol by 4-substituted-1,2,5-thiadiazole.[68] The most outstanding compound in this respect was XII, whose resolved base had an ED_{50} of .0066 mg/kg, as determined by inhibition of tachycardia in an isoproterenol-treated ganglion-blocked anesthetized rat.



R = 4-morpholino
R^1 = t-butyl

XII

The pyridine isostere XIII of INPEA (2-{p-nitrophenyl}-1-isopropylamino-2-ethanol) was found to act both as a partial agonist and a partial antagonist in isolated guinea pig atrial strips against isoproterenol challenge.[69] On the other hand, the 4-pyridyloxypropanolamine derivative XIV was a pure antagonist (pA_2=7) and was ten times as potent as its p-nitrophenoxy isostere.



XIII, R = $CH(OH)CH_2NH$-i-Pr
XIV, R = $OCH_2CH(OH)CH_2NH$-i-Pr

Perhexilene maleate[70] (Pexid) was reported to be effective clinically in reducing the frequency of anginal attacks.[71] Presumably, studies for acute and chronic treatment of angina pectoris with perhexilene will continue.

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Chapter 9. Antithrombotic Agents

Roy G. Herrmann and William B. Lacefield
Eli Lilly and Co., Indianapolis, Indiana 46206

This chapter summarizes the research reports of 1972 on all types of agents for the management of thromboembolic disease. The practice of discussing the three therapeutic approaches to thrombosis separately has been continued.[1] Space does not permit the review of important related work in surgery, diagnostic techniques and experimental models of thrombosis.

Platelet Aggregation Inhibitors

A conference on platelets and their role in hemostasis has provided an excellent review of platelet biochemistry, physiology and function.[2] The shadow-casting technique for whole platelets has afforded observations of the very early events produced on the platelet surface by aggregating agents. A variety of aggregating agents (ADP, thrombin, collagen, etc.) appear to produce a common sequence of changes leading to aggregation. Inhibitors appear to fall into several classes depending on which of the steps in the aggregation sequence they block.[3]

Cyclic AMP - It is generally accepted that agents which increase platelet cAMP (either by stimulating adenyl cyclase or by inhibiting phosphodiesterase) will inhibit platelet aggregation and that agents which induce or enhance aggregation decrease the cAMP level.[4] However, thrombin, in concentrations which induce platelet aggregation, has been reported to increase cAMP levels.[5] The mechanism by which cAMP exerts its effect on platelets is not completely understood. However, the discovery that it stimulates a protein kinase responsible for activating phosphorylase and inactivating glycogen synthetase may be pertinent.[6] A cAMP-binding protein with protein kinase activity has been isolated from the soluble fraction of homogenized platelets.[7] The observation that cAMP phosphodiesterase from human platelets is strongly inhibited by dipyridamole, an inhibitor of platelet aggregation, and only slightly inhibited by psychotropic drugs, whereas the reverse is observed with the brain enzyme, has led to the proposal of different isoenzymes. Different electrophoretic patterns for the phosphodiesterases from these tissues support this proposal.[8] The role of prostaglandins in cellular biology was the subject of a recent conference.[9] The powerful inhibitory activity of PGE_1 on ADP induced aggregation is probably due to its stimulatory effect on adenyl cyclase. PGE_2 inhibits intracellular cAMP accumulation induced by PGE_1 in rat platelets. PGE_2 counteracts PGE_1 inhibition of platelet aggregation and shape change.[10] PGE_2 has been reported to exhibit a weak PGE_1-like inhibitory effect on the primary phase of aggregation followed by a stronger stimulatory effect on the second phase.[11]

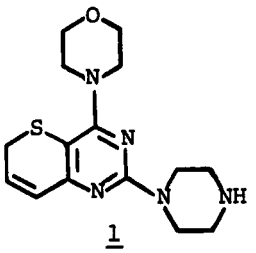
Aspirin - The antithrombotic effect of aspirin continues to be the subject of many investigations. It has been reported effective in reducing thrombus formation on catheters placed in the inferior vena cava of cats,[12] in reducing the incidence and extent of occlusive platelet aggregation in dog arteries following endothelial injury,[13] and in inhibiting the circulatory

and ventilatory effects of protamine induced platelet aggregation in dogs.[14] It was without effect on platelet thrombosis at sites of biolaser induced endothelial injury in rabbit ear chamber preparations[15] or on the incidence or size of coronary and femoral thrombi induced in dogs by use of a catheter electrode.[16]

Clinical investigations have shown that 1.5 g of aspirin reduced the incidence of thromboembolic complications following major hip surgery.[17,18] Another large-scale study showed 600 mg of aspirin to be without effect on the incidence of post-operative deep-vein thrombosis.[19] Aspirin abolished multiple episodes of transient monocular blindness secondary to retinal emboli,[20] and in combination with dipyridamole reduced the incidence of post-operative deep-vein thrombosis,[21] and reduced thrombus formation on dialyzer membranes during dialysis.[22]

Sulfinpyrazone - This agent has been reported to prolong survival of renal allografts in dogs[23] and to reduce the incidence of recurrent venous thrombosis associated with carotid artery stenosis in man.[24] At present a double-blind clinical trial in a national cooperative study is underway in Canada to evaluate the effectiveness of aspirin and/or sulfinpyrazone for antithrombotic utility.[25]

Pyrimidopyrimidines - This class of agents (dipyridamole, RA 233, RA 433, VK 744) inhibit the primary phase of ADP induced platelet aggregation apparently by virtue of their inhibitory effect on phosphodiesterase.[26] Dipyridamole, 100 mg q.i.d., normalized the decreased platelet survival in patients with arterial disease, prosthetic heart valves, arterovenous cannulas, prosthetic aortic grafts, and other vascular disorders. Aspirin, 4 g/day, had no effect on the decreased platelet survival in arterial thrombosis and prosthetic heart valves but increased the effectiveness of dipyridamole.[27] VK 744 inhibited platelet aggregation and increased the kaolin-stypven time in man, but the trial was discontinued because of side-effects.[28] Compound 1, VK 774, has been reported to be the most active *in vitro* inhibitor of ADP-induced platelet aggregation of this class.[29] It also exhibited potent antithrombotic action in injured rabbit arteries.[30] However, side-effects caused the clinical trial of VK 774 to be discontinued also.[31]



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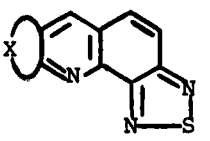
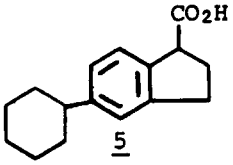
Dextrans - The mechanism by which the dextrans exert their antithrombotic effect is unresolved. It has been suggested that changes in fibrin structure, apart from dilution of coagulation factors and increased venous flow, are as important as the decreased platelet adhesiveness.[32] Several reports showing prophylactic dextran to be effective for the treatment of postsurgical thromboembolic complications have appeared.[33,34] It has been used with success in treating pregnancy-associated thrombophlebitis.[35]

New Agents - Reports of new platelet aggregation inhibitors continue to appear at a rapid rate. The acridines 2, 3 and the quinoline 4 were



2 X=(CH=CH)$_2$
3 X=(CH$_2$)$_4$
4 X=(CH$_2$)$_3$

tested for their *in vitro* inhibition of ADP induced rabbit platelet aggregation. Compound 2 inhibited aggregation at 10^{-5}M, whereas 3 and 4 were inactive at 10^{-4}M.[36] BL-2365 5 and fenoprofen 6 are both new non-steroidal anti-inflammatory agents. BL-2365 was nearly as potent as aspirin *in vitro*, and significant inhibition was observed in dogs at doses of 5 mg/kg and higher. Oral dosing prolonged bleeding time in mice and reduced the lethality of intravenous bacterial endotoxin injection in rats. Intravenous infusion modified the endotoxin induced hypotension and platelet aggregation in dogs.[37] Fenoprofen 6 was found to be a more effective



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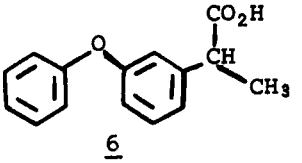

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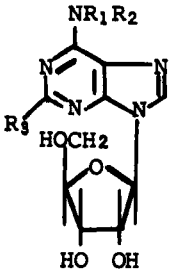
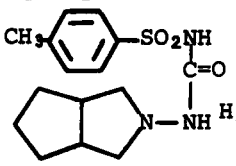
inhibitor of collagen induced platelet aggregation than either aspirin or phenylbutazone. Oral dosing reduced collagen induced aggregation in rabbits and guinea pigs and increased bleeding time in mice. It was slightly more effective than aspirin in reducing the dry weight of thrombi formed in an extracorporeal shunt in rabbits.[38]



7 R$_1$=R$_3$=H, R$_2$=OH
8 R$_1$=H, R$_2$=OH, R$_3$=NH$_2$
9 R$_1$=R$_2$=H, R$_3$=NH$_2$

A series of 5′, 2, 6 and 8 substituted adenosines were tested against ADP induced rabbit platelet aggregation. The hydroxylamines 7, 8 were 5-10 times as potent as adenosine and did not lose their activity after 40 min. incubation in plasma. Compound 9 was as active as adenosine.[39] A new oral hypoglycemic agent (S1702) 10 produced a decrease in platelet stickiness as measured by the Salzman glass bead technique 4 hrs after an oral dose of 10 mg/kg.[40] It also effected a significant decrease in platelet stickiness in 100 diabetic patients.[41] Both furosemide 11 and nitrofurantoin 12 were reported to be competitive inhibitors of ADP induced aggregation. The concentration of 11 required to inhibit aggregation *in vitro* could not be achieved *in vivo* with customary doses. With 12, *in vivo* activity was obtained.[42,43] A structure-activity relationship of a series of lactamimides led to the selection of RMI 7822, 13, which was found to inhibit human platelet aggregation induced by ADP, thrombin, epinephrine and serotonin with minimal release of PF3.[44]



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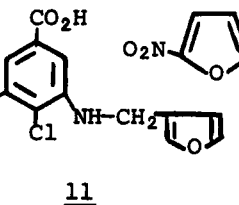

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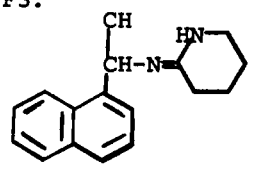

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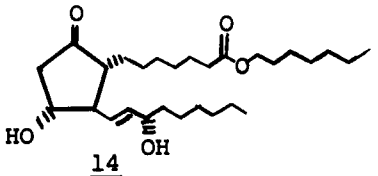
Omega-homo-PGE$_1$ heptyl ester, 14, has been claimed to be only slightly less potent than the corresponding free acid which is a more potent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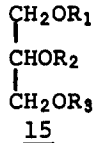
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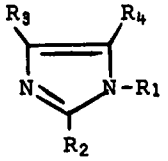
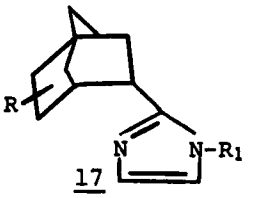

16

inhibitor of platelet aggregation than PGE_1. Further, 14 is claimed to be free from hypotensive and intestinal-contraction side-effects and to have 10 times the duration of action of omega-homo-PGE_1.[45] A series of prostaglandin glycerides, 15, in which the R groups may be H, stearoyl, or palmitoyl, with at least one R being a prostaglandin residue, were reported to have the expected antithrombotic activity of the parent prostaglandin, but with much increased biological half-lives.[46] A large series of di-(furyl,thienyl, pyridyl or substituted phenyl)-trifluoromethyl imidazoles 16 were patented as anti-inflammatory agents and platelet aggregation inhibitors. The most active compound (R_1=H, R_2=CF_3, R_3=R_4= 4-$CH_3OC_6H_4$) also inhibited collagen induced aggregation (human, dog, rabbit) *in vitro* at concentrations of 10^{-6} to 10^{-7}M. Inhibition of platelet function was observed in dogs after 25 mg/kg orally.[47] The claimed utility of a series of 5-endo(2-imidazolyl)-bicyclo(2,2,2)octenes 17[48] and a series of thieno(3,2-d) pyrimidines 18[49] was reported to be inhibition of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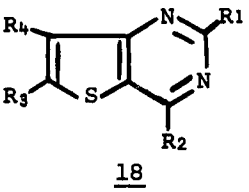
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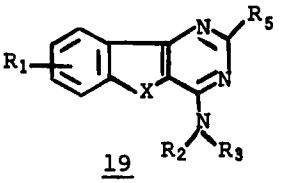
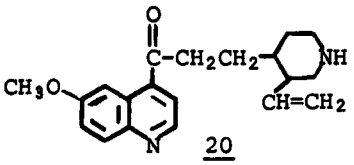

19

platelet aggregation. In another series of thienopyrimidines 19, *in vitro* inhibition was observed with concentrations of 10^{-5} to 10^{-6}M. Oral doses of 10 to 200 mg effectively prevented thrombus formation.[50] The vasodilator viquidil, 20, was reported to inhibit ADP, epinephrine, and collagen induced human platelet aggregation at concentrations of about 10^{-4}M. The long-acting orally effective coronary vasodilator, lidoflazine, was found to inhibit ADP induced platelet aggregation in a manner comparable to that of dipyridamole. Cinnarizine was slightly less potent.[51]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Anticoagulants

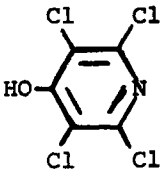
The prophylactic value of anticoagulants derives from their ability to inhibit intravascular fibrin formation. Heparin and the coumarins have long been used with advantage. The most commonly observed side-effect with these anticoagulants has been hemorrhage. Some promise that this side-effect can be minimized with heparin has been shown in the recent work with low-dose heparin therapy. This approach appears to be effective, safe and well tolerated.[52-54] It has been shown that heparin concentrations as low as 0.1 unit/ml, a level which can be attained by

the low-dose regimen, enhances the activity of a naturally occurring inhibitor of activated factor X.[55,56]

The anticoagulant activity of the coumarins has been attributed to their ability to block the synthesis of vitamin K. Recent work has shown that vitamin K is required for the synthesis of the active procoagulant factors II, VII, IX, and X. During coumarin treatment, inactive forms of these factors are synthesized, resulting in anticoagulant activity.[57,58]

The procoagulant enzymes prepared from the venom of snakes (arvin from the Malayan pit viper, reptilase from the South American pit viper and the enzyme from the North American diamondback rattlesnake) act directly on fibrinogen to form fibrin.[59,60] The fibrin formed by these enzymes is abnormal and is lysed more readily by plasmin. Reptilase, in contrast to thrombin, induces only minimal and delayed platelet aggregation without activation of the release reaction. Therefore, defibrinogenation can be produced without the hazard of thrombocytopenia.[61] Two anticoagulant proteins have been isolated, one from the venom of *Vipera aspis* and the other from *Vipera berus*. They appear to interfere with prothrombinase formation and do not seem to be enzymatic themselves. They may inhibit the phospholipids involved in the clotting process.[62]

A series of chloropyridinols has been reported to have coumarin-like anticoagulant activity. The oral activity of 21, the most effective compound in the series, showed a peak effect 48 hrs. after dosing. It was less potent than warfarin and vitamin K was observed to antagonize its action.[63]



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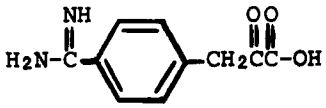


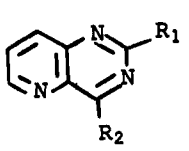
22

A number of aromatic amidines (activated esters and sulfonyl fluorides) have been reported to possess antithrombin activity. These compounds appear to act by acylation of the serine residue of thrombin.[64-67] The lethal effect of thrombin infusion in experimental animals was reduced by 4-amidinophenyl pyruvic acid 22.[68] A series of peptides involving arginine or lysine and sarcosine were also reported to have antithrombin activity. The most interesting of these was tosyl-L-arginylsarcosine because of its longer duration of action.[69]

Glucuronolactone, 1.5 g/kg p.o., was reported to result in a significant increase in whole blood clotting time in rats. It had a rapid onset (30 min) and the effect persisted for 6 days after a single oral dose. There were no apparent side-effects. A metabolite, 1,4-saccharolactone, was also reported to be active.[70]

A series of pyrimido(3,2-d)pyrimidines was reported to have anti-

coagulant activity. Compounds 23-26, at 10 mg/kg p.o., were found to increase bleeding time 100% or more. These agents also decreased platelet adhesiveness.[71]



	R_1	R_2
23		
24		
25		
26	$N(CH_2CH_2CH_2OH)_2$	

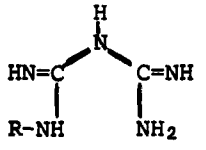
Fibrinolytic Agents

Numerous clinical studies have been and are still being conducted in an effort to evaluate the therapeutic effectiveness of the activated fibrinolytic system in thrombotic conditions.[72-76] The results of these studies are still controversial. The benefit obtained appears to be relatively small and hemorrhagic side-effects are such that the availability of good hospital facilities for the studies is mandatory. The major clinical effort involves the use of streptokinase. The recent availability of high-purity streptokinase appears to have resulted in a decrease in the incidence of antigenic side-effects. This development has resulted in increased interest in the use of streptokinase, especially in the United States. Both urokinase and streptokinase appear to be of value in the treatment of pulmonary thromboembolism and venous thrombosis. Their effectiveness in arterial thrombosis is probably less than in venous thrombosis and fibrinolytic therapy in myocardial infarction appears to be of little value.[77,78] However, streptokinase therapy has been successfully applied in 26 of 66,[79] and in 7 of 20, cases of arterial thrombosis.[80] In dogs, it has been demonstrated that plasminogen activation requires a much lower dose of streptokinase when combined with heparin.[81]

The proteolytic enzyme from *Aspergillus oryzae*, brinolase, has also received attention. It is non-pyrogenic, non-antigenic and has a direct thrombolytic effect of its own. Endogenous inhibitors of this enzyme appear to be present in the blood and, if administered in doses which do not exceed the inhibitor capacity, thrombolysis can be achieved without general proteolysis.[82-84]

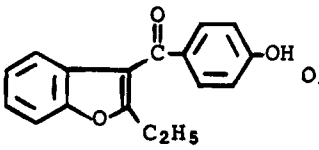
The anabolic steroid, stanozolol, was found to enhance spontaneous fibrinolysis in 34 myocardial infarction patients. This effect was not enhanced by the simultaneous administration of phenformin 27 as is the case with ethyloestrenol.[85] The biguanides 27 and buformin 28 may be fibrinolytic by direct activation of plasminogen and by inhibition of antiplasmin.[86,87]

In a series of benzofurans, benzarone, 29, was found to possess



27 R=$C_6H_5CH_2CH_2$

28 R=*n*-C_4H_9



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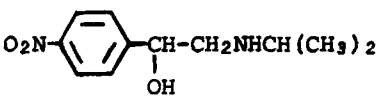
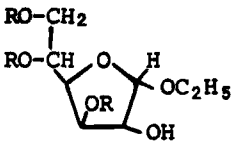


30

fibrinolytic activity in the dog. Either intravenous or oral treatment of an experimental occlusive thrombus resulted in complete functional recanalization of the femoral artery.[88]



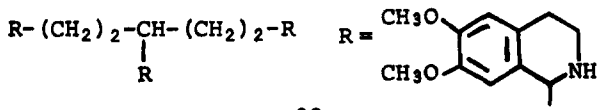
31 R=$C_6H_5CH_2$

The β-blocking agents, oxyprenolol and 30, were found to increase fibrinolytic activity in man at therapeutic dosage but did not show activity *in vitro*.[89]

The anti-inflammatory agent tribenoside 31 was found to stimulate fibrinolytic activity both *in vitro* and *in vivo* in the rat and in human plasma. The *in vivo* effect in man is currently under investigation.[90] Flufenamic acid enhances the fibrinolytic activity of urokinase and that of endogenous activator by inhibiting the antiplasmins.[91]

A synthetic, heparin-like, sulfated polyanion (SP 54) was shown to induce fibrinolytic activity in 12 patients after dosing either i.v., i.m. or orally.[92] The sulfhydryl inhibitor, *p*-chloromercuribenzoic acid (*p*-CMB), when added *in vitro* during clot formation resulted in clots soluble in monochloroacetic acid and urea solutions. The use of *p*-CMB alone produced no lytic activity in dogs with experimental occlusive arterial thrombosis but when given together with plasmin enhanced the activity of this enzyme.[93]

A series of tetrahydroisoquinolines related to EN 1661 was reported to have substantial fibrinolytic activity. The most active compound in that series was EN 3047A 32. The fibrinolytic ED_{50}, as measured by the



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dilute whole blood clot lysis time, was 0.02 mg/kg i.p. in rats and 25 mg/kg p.o. in rats and dogs. The LD_{50} was 57 mg/kg i.p. and 325 mg/kg p.o. in rats.[94]

Comment

Anticoagulants are more effective in the treatment of venous thrombosis than arterial thrombosis. While anticoagulants might not prevent

the formation of a platelet dominated thrombus in the arterial circulation, they certainly can inhibit the stabilization and extension of that thrombus. Successful prophylaxis of arterial thrombosis must deal with the etiologic role of the platelet. The value of platelet function inhibitors in venous thrombosis will be reflected by the extent to which platelets are involved in the formation of those thrombi. Certainly, within the circulatory system there are regions of stasis in which fibrin formation would be virtually the sole participant in thrombosis and other regions of high hemodynamic activity where the platelet nidus alone could block the vessel. Between these two either/or extremes, there are those situations in which fibrin formation and platelet aggregation occur simultaneously, each supporting the other. The suggestion has been made that 'drug cocktails' employing a mixture of agents which inhibit platelet function by different mechanisms (e.g., agents which block the release reaction with agents which block phosphodiesterase)might be clinically advantageous.[95] In this review we have cited reports of the effective combination of aspirin and dipyridamole[21] and of heparin with streptokinase.[61] As the degree to which fibrin formation and platelet aggregation participate in various clinical types of thrombosis becomes better known and experience is gained with fibrinolytic agents, the application of 'tailor-made' mixtures of anticoagulants, fibrinolytics, and anti-aggregation agents could provide extremely effective prophylactic and therapeutic measures.

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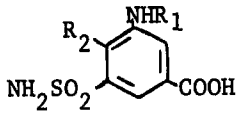
Chapter 10. Diuretics

Gerald R. Zins, The Upjohn Company, Kalamazoo, Michigan

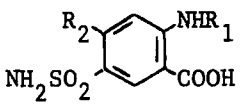
Considerable progress has been made to improve diuretic therapy over the past 15 years. Early in this era, the discovery of thiazides and, subsequently, phthalamidines overcame the disadvantage shared by carbonic anhydrase inhibitors and organomercurials of rather severe acid-base imbalance which resulted in refractoriness to their diuretic action. However, many cases of edema could not be resolved with these drugs and, with their long-term use, problems of negative potassium balance, uric acid retention and glucose intolerance emerged. Later, the "high efficacy" diuretics, ethacrynic acid and furosemide, virtually eliminated occurrence of drug-resistant edema and added the dimension of effective treatment of renal failure but continued to lower plasma potassium and elevate plasma glucose and uric acid and introduced hypochloremic alkalosis and a risk of ototoxicity as new concerns. The diuretic bases, triamterene and amiloride, and the aldosterone antagonist, spironolactone, also were developed during this period but, while these drugs spared potassium and possibly were free of hyperglycemic and hyperuricemic properties, they presented the new risk of potassium retention. Thus, the "ideal diuretic", as a single chemical entity, presently does not exist, and efforts to improve therapy continue.

Chemistry and pharmacology of new diuretics. The most extensive developments in the chemistry of diuretic agents during the past two years come from the work of Feit and colleagues who evaluated the influence of isomerism and new substituents on the diuretic properties of the aminosulfamylbenzoic acid molecule. Several N-substituted 3-amino-4-halogeno-5-sulfamylbenzoic acids (I) were synthesized and their diuretic actions were compared to furosemide-like isomers from the 2-amino substituted anthranilic acid series (II)[1]. A number of type-I compounds produced "high efficacy" diuresis and natriuresis. The most potent of these were Ia and Ib which increased sodium, chloride and potassium excretion in dogs as a continuous function of dose in much the same fashion and with the same potency as furosemide. Also analogous to furosemide, the activity was early in onset and brief in duration. The principle difference between the 3-amino and 2-amino derivatives was that a variety of N-substituents (benzyl, n-amyl, isoamyl, n-butyl, 2-methylfuryl) uniformly enhanced activity in the former series whereas 2-methylfuryl uniquely increased potency of the anthranilic acid molecule. By varying substituents in the 4 position of I, the generalization that benzenesulfonamide diuretics require halogen or pseudohalogen adjacent to the sulfonamide group for activity was evaluated[2]. Fixing R_1 as benzyl, several non-halogen moieties dramatically enhanced potency (compounds Ic - Ie) when compared to the corresponding bromine and chlorine substituted derivatives. When R_2 was phenoxy, compounds with various substituents in R_1 (Ie - Ih) were equipotent to each other and 40 times as potent as furosemide as natriuretic and kaliuretic agents. Saturating or substituting the phenyl ring or shortening the n-butyl chain of R_2 sharply diminished potency.



I

	R_1	R_2
Ia	n-butyl	Cl
Ib	n-butyl	Br
Ic	$CH_2C_6H_5$	NHC_6H_5
Id	$CH_2C_6H_5$	SC_6H_5
Ie	$CH_2C_6H_5$	OC_6H_5
If	n-butyl	OC_6H_5
Ig	$CH_2CBr{=}CH_2$	OC_6H_5
Ih	$CH_2C{=}CHCH{=}CHO$	OC_6H_5



II

	R_1	R_2
IIa	$CH_2C{=}CHCH{=}CHO$	Cl
IIb	$CH_2C{=}CHCH{=}CHO$	OC_6H_5
IIc	$CH_2C_6H_5$	OC_6H_5
IId	$CH_2C_6H_5$	SC_6H_5
IIe	$CH_2C_6H_5$	Cl

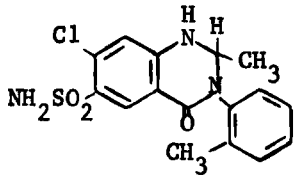
If - bumetanide

IIa - furosemide

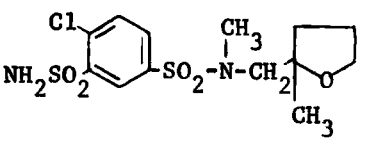
Non-halogen substitution of position 4 in the anthranilic acid series similarly increased diuretic potency[3]. Thus, IIb had 20 times the potency of furosemide and IIc and d were at least 10-fold more potent than IIe on the basis of volume and electrolyte excretion in the dog. Consistent with prior evidence of a unique activating property for the 2-methylfuryl group as R_2 in the furosemide series, IIb was 10-fold as potent as IIc. The activating effect of 4-substitution with either OC_6H_5 or SC_6H_5 in both the 2-amino and 3-aminosulfamylbenzoic acid series was not applicable to other sulfonamides, however. None of the derivatives of chlorothiazide, hydrochlorothiazide, quinethazone or clopamide, in which these groups replaced halogen ortho to the sulfonamide moiety, exerted diuretic effects[4].

The pharmacology of bumetanide (If) has been carefully examined in animals[5] and man[6]. In the dog, bumetanide is a highly effective diuretic which resembles furosemide precisely in respect to rapid onset and short duration of action, high efficacy, isosmotic urine, absence of bicarbonate excretion, and urinary sodium:potassium ratios. However, bumetanide has a milligram potency 50 to 100 times that of furosemide in the dog and, like many other diuretics including furosemide which inhibit electrolyte reabsorption in the ascending limb of the loop of Henle[7], is appreciably less effective in the rat. Clinically, bumetanide could be distinguished from furosemide only on the basis of its 40-fold greater milligram potency. Potassium loss was evident from single dose studies and plasma uric acid rose with repetitive treatment.

Metolazone, a sulfamoylquinazolinone, was described previously[8] as closely resembling hydrochlorothiazide in its renal actions. Subsequent animal studies fortify this conclusion by providing evidence of inhibited sodium reabsorption in the cortical diluting segment[9] and negative potassium balance during chronic administration[10]. In man, metolazone, like hydrochlorothiazide, inhibits free water clearance (CH_2O)[11-13] but not solute free water reabsorption (TcH_2O)[12,13] which denotes reduced solute transport in the cortical part of the distal tubule but not in the medullary ascending limb. This drug may also diminish proximal tubular reabsorption to a limited extent since it tends to increase TcH_2O[12,13] and the urinary clearance of proximal marker ions, i.e. phosphate, calcium and bi-



metolazone

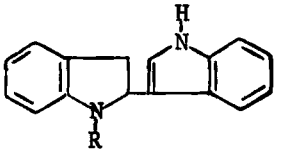


mefruside

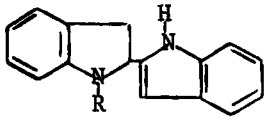
carbonate[12]. This proximal tubular action may be responsible for the limited effectiveness with which very high doses of metolazone have been used to treat chronic renal failure[14]. During chronic treatment in hypertensives, blood pressure and plasma K^+ fell while plasma urate, HCO_3^- and nitrogenous materials rose in much the same manner that they do with a thiazide diuretic[15]. Studies with mefruside, another sulfonamide-containing diuretic, also showed a close correlation with thiazides in terms of both desirable and undesirable effects in hypertensive patients[16-18].

In contrast to the seemingly inevitable potassium losing properties of organic acid diuretics, organic bases generally produce potassium retention. The clinical role for such agents until now seems largely as adjunctive therapy with other diuretic drugs. These compounds tend to be very effective diuretics in the rat but less effective or ineffective in dogs, and most of the evidence in man agrees with the canine findings. Thus, studies cited earlier[8] as well as recent work[19,20] indicate that amiloride suffers deficiencies as independent therapy but very appropriately potentiates the natriuretic effect and antagonizes the hypokalemic action of acidic diuretics when they are given in combination.

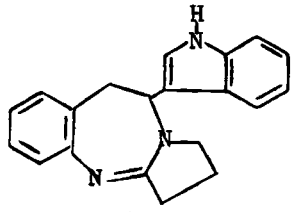
New potassium sparing organic base diuretics continue to be described. Wu *et al.*[21] have recently synthesized a series of diuretic, natriuretic 1-imidoyl-2-(2- and 3-indolyl) indolines (III and IV), many of which either failed to influence urinary potassium excretion or caused potassium



III



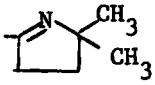
IV



V

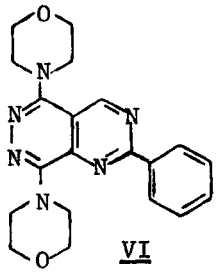
IIIa R = $CH{=}NC(CH_3)_3$

IIIb R = 

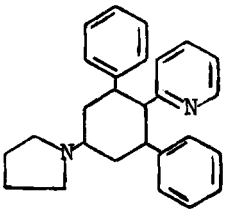
IVa R = 

retention. Compounds in each series increased volume and sodium excretion in rats to a greater extent than did maximally effective doses of a thiazide. An imidoyl moiety at the 1 position (R), either in a cyclic or noncyclic conformation, was essential for diuretic activity. In a homologous series, diuretic activity increased as the carbon attached to the imidoyl nitrogen progressed from primary to secondary to tertiary substitution. Substitution in the 5 position of the indoline nucleus exerted variable effects on activity, not accounted for by electronic influences. Several compounds (IIIa,b; IVa; V) are under continuing investigation.

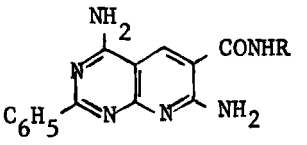
Additional work goes on with multiple, nitrogen-containing ring systems. VI is described as having natriuretic efficacy in rats and dogs comparable to thiazides but lacking a kaliuretic action[22]. This agent affects neither dilution nor concentration of urine, thus being different in tubular site of action from thiazides, carbonic anhydrase inhibitors and "loop diuretics", and, therefore, additive in effect with many of these other compounds. VI was inactive in adrenalectomized rats but responsiveness was restored by administering cortisol. This activity profile is similar to that of VII which was described as ineffective in adrenalectomized rats[23] and which recently was found to antagonize the potassium losing action but to add to the natriuretic action of thiazides and other diuretics[24]. Yet another multiple, nitrogen-containing ring system (VIII), a 5-deaza isostere of the pteridinecarboxamide series, yielded compounds inactive as diuretics[25].



VI

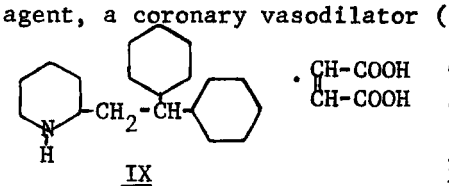


VII



VIII

Diuretic actions have also been described for a miscellaneous group of compounds, most of which act through mechanisms other than direct inhibition of tubular ion transport. Isoproterenol[26], an adrenergic beta receptor agonist, and histamine[27] increased volume, sodium and chloride excretion when infused into the renal artery of dogs. Both agents enhanced glomerular filtration rate (GFR) and renal blood flow (RBF) as a result of renal vasodilitation and these changes were thought to be responsible for the heightened salt and water excretion. The effect of infusion of acetylcholine[28,29], prostaglandin E_1[28], or bradykinin[29] into the renal artery of dogs continues to be investigated and earlier contentions that these agents cause diuresis by suppressing proximal tubular salt and water reabsorption have been supported. Bradykinin and acetylcholine may also limit electrolyte transport in the ascending limb[29]. Still another vasoactive agent, a coronary vasodilator (IX), increased volume, sodium and potassium excretion when given orally to rats[30]. This agent was less potent but nearly as efficacious as hydrochlorothiazide. Whether these changes were secondary to altered renal hemodynamics or the result of inhibition of tubular ion transport has not been disclosed. Intravenous guanethidine and reserpine also increased salt and water excretion in dogs as a result of catecholamine release and the accompanying elevation in blood pressure[31]. Finally, the antibiotic, amphotericin B, increased sodium and potassium clearance while decreasing GFR and RPF in dogs[32]. This agent appeared to directly inhibit electrolyte reabsorption in the cortical distal tubule.



IX

Cellular mechanisms of diuretic action. The most thoroughly researched

mechanism in recent years by which diuretics might act to facilitate urinary electrolyte and water excretion is inhibition of the Na^+-K^+ activated ATPase of renal tubule cells. This enzyme couples hydrolysis of the terminal phosphate of ATP with the active transmembrane movement of ions. Na^+-K^+ ATPase recently has been localized to the infoldings of the basal (antiluminal) plasma membrane of the renal tubular cells[33]. In this location, according to one view[34,35], Na^+ ions inside the cell activate phosphorylation of the enzyme by ATP which results in a conformational change in the protein and translocates Na^+ ions to the outside. Facing the outside, a K^+ dependent phosphatase catalyzes dephosphorylation which liberates Na^+ ions and results in a reverse conformational change with translocation of K^+ ions inward. A second "pump" mechanism, not involving inward transport of K^+, also has been proposed to account for net movement of $Na^+ + Cl^-$ ions and water outward[36], but this mechanism is challenged by others[37] since facile back diffusion of K^+ can readily explain net movement of solute and accompanying water to the outside.

The role played by Na^+-K^+ ATPase in ion and water reabsorption varies in different parts of the renal tubule. The enzyme is present in large amounts[38] and has been shown to participate extensively in ion reabsorption from the thick portion of the ascending limb of Henle's loop[39-42]. Thus, the administration of cardiac glycosides in quantities that appreciably inhibit the enzyme in the outer red medulla of the kidney results in parallel natriuresis and impairment of urinary concentration and dilution. Na^+-K^+ ATPase is also appreciable in distal convoluted tubules and probably has a reabsorptive function here[38], but it is relatively deficient in the proximal tubule where inhibition by cardiac glycosides fails to increase salt and water excretion[39-42]. Ethacrynic acid (EA) inhibits a ouabain-sensitive Na^+-K^+ ATPase fraction from renal cortex and red renal medulla in dogs in a dose-dependent manner and the duration of this *in vivo* effect corresponded with the short term natriuresis[43]. Giving EA after complete inhibition with ouabain failed to further enhance the natriuresis, which was viewed as evidence that both agents act on the same enzyme and that the diuretic action of EA is so explained[39,43]. The relative refractoriness of the rat to EA was also accounted for in terms of this mechanism since EA inhibition was readily reversible in this species but irreversible in the dog[43]. A disparity between the low concentration of EA in dog renal tissue following *in vivo* dosing and the appreciable inhibition of Na^+-K^+ ATPase which results, has been cited as evidence that the kidney concentrates the drug in a critical compartment[44]. That this probably occurs is suggested by the finding of energy-dependent uptake and concentration of EA by renal tubular cells[45]. Furosemide(F) also inhibited Na^+-K^+ ATPase in the ascending limb and distal convoluted tubule of the rat kidney, both anatomical sites of its diuretic action, but, consistent with near normal sodium reabsorption in the proximal tubule, did not affect the enzyme in this segment[46].

In spite of rather convincing evidence, not all investigators agree that inhibition of Na^+-K^+ ATPase accounts for the effects of EA and F on sodium excretion. In one study, neither drug inhibited this enzyme in a plasma membrane fraction obtained from kidneys of rats treated *in vivo*[47].

In another instance, there was a discrepancy between enzyme inhibition by EA and altered proximal tubular reabsorption[48]. Findings of this type have fostered the view that such agents act by interfering with ATP generation. For example, EA and F are alleged to slow renal glycolysis, not by reducing energy demand secondary to a direct inhibition of Na^+ transport but by inhibiting glyceraldehyde-3-PO_4 dehydrogenase[49]. EA and chlormerodrin also diminished O_2 uptake by rodent kidney slices incubated in sodium-free media, apparently ruling out direct inhibition of sodium transport as the mechanism[50,51]. Controversy exists here, however, since EA[52] and F[53] in toad bladder and dog kidney slices, respectively, inhibited O_2 uptake only when sodium was available for transport in the medium, which suggested reduced energy demand instead of supply. Interestingly, efforts are even being made to establish mechanisms such as interference with high energy phosphate generation as a basis for the diuretic action of carbonic anhydrase inhibitors[54,55], but there is little support for this possibility or for the possibility that thiazide-like diuretics work through any of these mechanisms.

Amiloride acts in the renal distal convolution and/or the collecting duct to inhibit Na^+ reabsorption and K^+ secretion. The mechanism has been studied primarily in the anatomically more simple toad bladder where the drug inhibits short circuit current and transepithelial Na^+ transport[56]. In the toad bladder, amiloride suppresses pyruvate oxidation[57] and O_2 consumption[58], both presumably secondary to Na^+ transport inhibition since no effect is seen in the absence of Na^+ at the mucosal surface. The intracellular Na^+ concentration[57] (or Li^+ which is also transported by the toad bladder[59]) falls. This is interpreted as an indication that amiloride diminishes the Na^+ permeability of the cell membrane facing the mucosal surface and thereby reduces the concentration of Na^+ entering the intracellular transport pool. This in turn should result in less Na^+ activation of transport Na^+-K^+ ATPase at the antiluminal membrane, less transcellular Na^+ transport, less ATP utilization, and less ATP generation[60]. The nature of the interaction of amiloride is not well defined although the presence of Ca^{++} appears necessary, and a ternary complex involving drug, Ca^{++} and receptor has been postulated[61,62]. According to one view, based on frog skin studies, triamterene acts similarly to amiloride in limiting Na^+ entry and diminishing the intracellular transport pool[63]. A second view is that these drugs act at different sites on the Na^+ transport mechanism[64] although the difference is not well defined. The reduced potassium excretion that both agents produce probably results from reduced, Na^+ transport-dependent electronegativity in the fluid bathing the mucosal (analogous to luminal) surface which diminishes the electrochemical gradient favoring K^+ secretion[64].

Cyclic AMP and the enzymes which produce (adenylcyclase) and destroy it (phosphodiesterase) have been recognized for several years to be involved in the renal actions of antidiuretic hormone (ADH) and parathyroid hormone (PTH). Recently this ubiquitous system has been proposed as the receptor for an exogenous diuretic as well. The compound believed to act here is an indolyl indoline (IIIa) which quite selectively inhibits the cyclic AMP phosphodiesterase (PDE) of rat and guinea pig kidney, and there-

by raises the concentration of cyclic AMP in renal cells[65]. Evidence cited in support of this mechanism includes the finding that certain other diuretics, i.e. thiazides[66] and theophylline[67], also inhibit this enzyme in renal tissue. Whether this mechanism is applicable remains to be established. In this connection it is useful to examine knowledge about the location and the role of the cyclic AMP system in renal function. The synthesizing enzyme, adenyl cyclase, is localized principally in the basal cell membrane of tubular endothelial cells, essentially the same site occupied by Na^+-K^+ ATPase[68,69]. A number of cyclase enzymes with differential responsiveness to known activators are found in various tubular segments. The enzyme from the renal medulla is preferentially activated by ADH whereas PTH selectively activates cyclase from the cortex (mostly proximal tubular cells)[68,70,71]. A number of other activators including isoproterenol, fluoride and calcitonin appear to show no anatomical preference[68]. It is interesting to note, however, that in the reported instances when the effect of augmented cyclic AMP levels has been imposed on the kidney, either by infusion of cyclic or dibutyryl cyclic AMP[72-74] or by administration of activators of renal cyclases[73-75] or cyclases elsewhere in the body[74-76], Na^+ reabsorption has been suppressed only in the proximal tubule. Moreover, elevation of cyclic AMP in more distal tubular elements by ADH does not affect sodium excretion. While these are not conclusive arguments that this system lacks the capacity to alter Na^+ reabsorption in the distal nephron, the possibility is strongly suggested. In any case, it seems unlikely that inhibition of renal PDE by thiazides explains their natriuretic effect since these agents inhibit sodium transport principally in the distal tubule[8]. Accordingly, the prospect that compound IIIa enhances salt and water excretion by inhibiting renal PDE would be strengthened if this agent were found to act predominantly in the proximal tubule.

Natriuretic hormone. The concept of a natriuretic hormone received its greatest impetus in 1961 when DeWardener et al.[77] showed that the natriuresis which accompanied isotonic saline expansion in dogs could not be accounted for by changes in filtration rate (GFR) or mineralocorticoid secretion and, furthermore, that sodium excretion by a kidney from a second animal could be increased by infusing it with blood from the expanded dog. Subsequently, many investigators pursued this question and, as a result, not one but a variety of newly defined factors which regulate sodium excretion by the kidney have been elucidated. Among these, a blood borne chemical substance or hormone remains of great interest since it (they) now seems certain to exist and since replicatable methods have been dedeveloped to demonstrate its activity.

Convincing evidence of the existence of sodium transport inhibitory materials in either blood or urine has come from a number of sources. One such material, obtained from either urine or plasma of salt-loaded man or sheep, was considered protein-like and had a molecular weight between 10,000 and 50,000[78,79]. When administered into the circulation to either diabetes insipidus or normal hydropenic rats, a delayed, modest increase in Na^+,K^+ and water excretion occurred. Urine of volume expanded hypertensive subjects provided an especially rich source for this type of molecule[80]. A small polypeptide, which enhanced sodium excretion in the rat

and cat and inhibited short circuit current in frog skin, was also found in human plasma[81]. This material was more abundant in blood coming from the brain than from the periphery and its release was facilitated by temporarily occluding the carotid artery or by perfusing the brain with oxytocin. The same or a similar substance, which inhibited short circuit current and lowered the potential difference across toad bladders, was also found preferentially in jugular vein blood of saline loaded dogs[82]. Its MW was probably less than 1000. Yet a second small MW substance was obtained from serum of patients in chronic renal failure[83-85]. This material had a MW of less than 1000, resisted boiling, freezing and digestion with proteolytic enzymes or acid but was inactivated by alkali. Modest natriuretic activity was produced by parenterally dosing rats, and inhibition of kidney slice PAH uptake and frog skin short circuit current also occurred.

Aside from general characterization, what might the chemistry of such a molecule be? The small polypeptide[81] has been thought to resemble oxytocin[86] because certain analogues of this hormone prevent the natriuresis produced in response to carotid occlusion. [2,4-Dileucine]oxytocin, an analogue without oxytocic or antidiuretic action, is an oxytocin antagonist and has the required natriuretic action[87]. Of even greater interest is the finding that both α- and β-melanocyte stimulating hormones, the former a naturally occurring polypeptide which appears in the pars intermedia of the pituitary gland and which disappears following hypertonic saline loading, are potent natriuretic agents in the rat[88,89]. Another possibility includes bradykinin, a known natriuretic substance, which is liberated from plasma kininogens by the enzyme kalikrein. It has been proposed that, in response to saline loading, kalikrein is elaborated by the kidney and produces natriuretic kinins intrarenally[90]. Certain non-polypeptide endogenous organic molecules have also been considered. Methylguanidine and guanidinosuccinic acid have been studied in this regard because they are found in increased amounts in the serum of uremic patients[91,92]. The former but not the latter was reported to increase sodium and potassium excretion in the rat. Prostaglandins also have been viewed as prospective natriuretic hormones, particularly PGE_2 and PGA_2 which are thought to be synthesized in the kidney[93]. PGE_2 presumably decreases sodium reabsorption in the distal tubule[94] which is viewed by many as a necessary site for the action of natriuretic hormone. Somewhat opposed to this notion, however, are the findings that the inhibition of Na^+ extrusion and K^+ uptake by isolated fragments of rabbit renal proximal tubules which one of the natriuretic hormones produces cannot be replicated with prostaglandins[95].

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Chapter 11. Agents Affecting Gastrointestinal Functions

Patricia W. Evers and Peter T. Ridley
Smith Kline & French Laboratories, Philadelphia, Pa.

In general, the statements made in the introduction to the previous biennial review of this area still prevail; the primary thrust of chemical and pharmacological research in this area is directed toward the control of acid secretion[1]. Approaches to this goal are based on ever-expanding knowledge of physiological mechanisms. This review attempts to reflect this state of the art.

Gastrointestinal Hormones

Reviews - The proceedings of an international symposium on these hormones has been published[2], and potential clinical applications have been proposed by Grossman[3]. Dupre[4] has reviewed the general literature; specific reviews have been published on the chemistry and biology of gastrin[5,6], on secretin[7], on intestinal hormones as inhibitors of gastric secretion[8], and on radioimmunoassay techniques[9].

Gastrin - When the heptadecapeptides, Gastrins I and II ("little gastrins"- LG - M.W. 2100) were first isolated from hog antral mucosa, it was speculated that eventually these substances might be found to be only a portion of a larger molecule, and indeed, a larger peptide, called "big gastrin" (BG - M.W. c 7000), has been identified. Now, "big, big" gastrin, with a molecular weight close to that of albumin, has been isolated[10]. LG is released from both BG and "big, big" gastrin by tryptic digestion; in this regard, the system of increasingly larger gastrin molecules resembles that already observed for other peptide hormones, e.g., insulin.

Structure-activity studies on gastrin continue to change our concepts of its interaction with receptor sites. The C-terminal tetrapeptide, Try-Met-Asp-Phe-NH_2, possessing all of the activities of the total molecule, had been proposed as the "minimal effective fragment" of gastrin, with a binding role assigned to the Try, Met, and Phe residues and a function role, to Asp. Lin[11], however, has demonstrated that the C-terminal tripeptide will induce most of the pharmacological actions of the tetrapeptide, although only at much higher doses. In addition, Trout and Grossman[12] have shown that a larger peptide (C-terminal octapeptide of CCK, OP-CCK) with the same C-terminal tetrapeptide sequence will stimulate gastric acid secretion and that Ala can be substituted for Asp in the OP-CCK molecule and still stimulate acid secretion.

The importance of the phenylalanine amide group for secretory activity was reconfirmed by McGuigan and Thomas[13] who showed that nonamidated human gastrin I is biologically inactive as well as almost immunologically unreactive with specific antibodies. The degree of secretory activity of a series of gastrin-related compounds depends to some extent on whether tyrosine is present at position 6 or 7 from the C-terminal end and whether

it is sulfated[14].

Tritsch, et al., prepared dimeric and cyclic analogs of gastrin[15,16]. On the basis of their studies of the biological activities of these substances, they suggested that both Asp residues of the dimer were functionally active, simultaneously occupying receptor sites on the chief and parietal cells, and that this necessarily required a linear conformation of the synthetic analog.

Important studies on the conformation of pentagastrin and the gastrin tetrapeptide have been reported[17,18]. Extrapolating from the results, Keir[17] has suggested some characteristics for possible gastrin inhibitors.

Jones synthesized several peptides related to the C-terminal tetrapeptide of gastrin by complementary reading of the genetic code; one of these, Z-Lys-Ile-His-Pro-NH_2, was found to have some inhibitory effect on gastric juice volume and acid and pepsin output in dogs after dosing for 3-8 days[19].

Several substances are known to stimulate gastrin release by local action on the antrum. Investigating molecular factors governing permeation of the antrum by gastrin releasers, Berkowitz, et al.,[20] found that the pH of the instilled solution affected permeability by its influence on the charge state of both the permeant compound and the mucosal surface. Smaller molecules (mol. wts. 46 to 75) permeated at a higher rate than choline (M.W. 121), but acetylcholine (M.W. 146) disappeared at the same rate as the smaller molecules. Some of the observations in vivo were confirmed by studies with a synthetic membrane and are in general agreement with the findings of Andersson and Elwin[21].

Cholecystokinin (CCK), Caerulein, and Related Peptides - Two techniques for radioimmunoassay of CCK have been reported, but both have distinct limitations[9]. It has been repeatedly demonstrated that CCK depresses gastrin stimulated acid secretion in dogs and man, probably by competitive inhibition[8]. Nakajima, et al.,[22] have now shown this inhibition in isolated Necturus gastric mucosa. In contrast, CCK did not inhibit pentagastrin in cats, demonstrating a species difference in response to this hormone[23].

The C-terminal octapeptide of caerulein, a peptide isolated from amphibian skin, closely resembles CCK in structure and activity. For both substances, optimal cholecystokinetic activity requires sulfated tyrosine in position 7 from the C-terminus. Italian researchers have now shown that the heptapeptide in which tyrosyl sulfate is replaced by p-sulfonylphenylalanine retains cholecystokinetic and other activities in animals and man at about 1/10 the potency of the total molecule[24].

Like gastrin and CCK with which it shares the common C-terminal tetrapeptide, caerulein appears to be a partial agonist for gastric secretion. In cats, caerulein and some analogs increased acid output in response to infusion of submaximal doses of pentagastrin, but depressed

acidity stimulated by maximal doses[25]. The most interesting aspect of these studies in cats was that infusion of caerulein and its analogs could protect against duodenal ulceration induced by infusion of pentagastrin or histamine[26].

Two other peptides (alytesin and bombesin) isolated from amphibian skin differ from caerulein in chemical structure, but resemble it in certain biological activities - contraction of gastrointestinal smooth muscle and stimulation of gastric acid secretion. The spectrum of activities of alytesin and bombesin, caerulein, and other peptides from the same sources have been reviewed by Anastasi[27] and Bertaccini[28].

Pyr-Glu-Arg-Leu-Gly-Thr-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
Alytesin

Bombesin differs in structure only by the presence of Gln instead of Gly in the penultimate position at the N-terminus.

Other Peptides - The tissues and secretions of the gastrointestinal tract seem to be an almost unlimited source of peptides which affect the activity of the organ system which produces them, as investigators continue to identify new substances.

The gastric inhibitory polypeptide (GIP) reported by Brown and coworkers can be classified as an enterogastrone based on its source and inhibition of gastric secretion and motility[1]. The complete amino acid sequence of GIP (43 residues) has now been determined and shows similarities to porcine secretin (27 residues) and glucagon (29 residues)[29]. This polypeptide may be responsible for some of the antisecretory activity of relatively impure preparations of CCK[30]. GIP has also been shown to increase secretion in the jejunum and ileum of dogs[31].

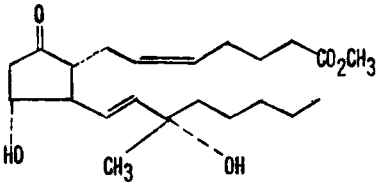
Brown and his group have also isolated from porcine duodenum a peptide which stimulates gastric motility and pepsin output but does not alter acid secretion. This substance which they have called "motilin" has been found to consist of 22 amino acid residues (M.W. c 2700). So far, it seems to be quite distinct from other fully characterized intestinal hormones[32].

The partial structure of vasoactive intestinal peptide (VIP, 28 amino acid residues) has been determined; this substance has chemical and biological features similar to secretin and glucagon[33].

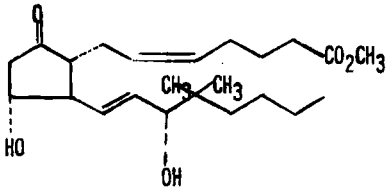
Additional studies on secretory inhibitors from thoracic duct lymph (chylogastrone)[34] and urine (urogastrone)[35] have been reported. Peptides from the pituitary are known to affect gastrointestinal function. Schapiro[36] has reviewed the literature on such activities of vasopressin, and Goodman and Hiatt[37] have reported a new substance, called "coherin", which they think has specific effects on motility.

Prostaglandins and Gastric Secretion

The synthesis of prostaglandin analogs over the past couple of years has resulted in several compounds with varying degrees of potency and oral activity. An 11-deoxy analog of PGE_1 (AY 22093)[38] was active parenterally in the rat; another analog, ($\Delta^{8(12)}$-13-PGE_1) (SC 24665)[39] suppressed secretion and ulceration in the rat when administered orally. Two analogs of PGE_2 have been cited recently[40,41] and have been shown to be very potent, possess a very long duration of activity, and have antisecretory activity in both rats and dogs. In addition, both compounds (15(S)-15-methyl PGE_2, methyl ester, and 16,16-dimethyl PGE_2, methyl ester) have been reported by Robert to prevent formation of gastric and duodenal ulcers in the rat[40].



15(S)-15-methyl PGE_2, methyl ester



16,16-dimethyl PGE_2, methyl ester

The mode by which prostaglandins inhibit gastric secretion is unknown. The pronounced influence that these compounds possess on cAMP has led to speculation that they act via the second messenger system. Because of the equivocal data concerning the role of cAMP in acid secretion[42], however, additional work is needed to ascertain this hypothesis.

Cyclic AMP and Gastric Secretion

Bieck[43] observed dose related increases in cAMP levels in secretion of humans or dogs following pentagastrin or histamine. Extensive studies by this investigator, showing reciprocal effects on cAMP with secretory stimulants and inhibitors, support the concept of a second messenger role. Correlations between mucosal levels of cAMP and HCl secretion were also found in the rat[44,45].

The results obtained following administration of exogenous cAMP are equivocal. In man and the dog[46] the nucleotide inhibited both histamine and pentagastrin induced secretion, while in another study[47] it stimulated secretion in man. Cyclic AMP administered intravenously to the rat stimulated secretion but the degree of response was dependent on the initial level of secretion[48]. This points up an additional problem in interpreting such data.

Accumulating evidence[49-51] suggests that histamine is capable of activating the gastric mucosal enzyme, adenyl cyclase. The action of gastrin on the enzyme is more in question. In vivo gastrin has been found to activate the enzyme, but when added to a gastric mucosal homogenate it did not affect adenyl cyclase[50]. In contrast to findings in other species, histamine does not stimulate cyclase nor did cAMP affect secretion in the dog[52].

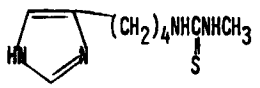
Gastric mucosal phosphodiesterase (PDE), an enzyme controlling metabolism of cyclic AMP, has also been the subject of several studies[52-54]. Sung and co-workers identified two cyclic-3',5'AMP diesterases which can be distinguished by K_m values. Both were theophylline sensitive and present in higher amounts in oxyntic than mucoid cells. Cyclic GMP, in contrast, was found primarily in the surface cells[53]. Theophylline inhibits PDE in vitro but not in vivo, a phenomenon that may be related to the state of the activator and inhibitor factors[54].

Antisecretory/Antiulcer Drugs

Two lengthy reviews on drugs for peptic ulcer disease have been published very recently[55,56]. In this section, we have updated the information in those reviews on certain drugs and have added others which seem to be of particular interest either chemically or biologically.

A Model - Few, if any attempts have ever been made to theorize broadly on the structural requirements of compounds which influence gastric acid secretion. Bravely, Bustard and Martin[57] have taken on this task, using as a basis for their approach, the known activities of several non-anticholinergic antisecretory/antiulcer compounds, as well as histamine and gastrin. Using structural data, qualitative conformational arguments, and theoretical conformation analysis (using extended Hückel theory), they have proposed the following requirements for activity: (1) the presence of two heteroatoms separated by 3.7±0.2Å in a low energy conformation; (2) one of the heteroatoms with its lone-pair electrons in a σ-type hybrid orbital and the other atom in a π-electron system. A test of fitness to this model with as yet undiscovered compounds is eagerly awaited.

Burimamide - This compound represents the first of a new pharmacological class - antagonists of the action of histamine at the H_2 receptor, as defined by Ash and Schild[58] and Keir[59]. In their approach to finding compounds of this type, Black, et al.,[60] initially took on the task of identifying histamine analogs which showed a separation of activity on H_1 and H_2 receptors, simultaneously confirming their existence. In the first publication of their landmark research, they have shown that 2-methyl histamine is more active on H_1 than on H_2 receptors and the reverse is true for the action of 4-methyl histamine. Having thus identified a rather specific agonist for the H_2 receptor, they proceeded to synthesize about 700 compounds in a search for specific antagonists, of which burimamide now is a prototype.

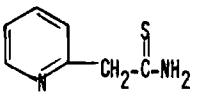


Burimamide

In vitro studies demonstrated competitive antagonism of burimamide for histamine at the H_2 receptors. In the anesthetized rat, burimamide inhibited (ED_{50} = 6.1 x 10^{-6} mol/kg iv) response to histamine infusion; it did not inhibit secretion induced by insulin or by direct vagal stimulation. Similar results were obtained in dogs where burimamide inhibited maximal histamine stimulation (ED_{50} = 1.9 x 10^{-5} mol/kg iv), but not carbachol stimulated secretion. Significant inhibition of pentagastrin and food stimulated secretion was also obtained. Subsequent studies in man

have shown inhibition of secretion stimulated by both histamine and pentagastrin at doses of 3.8 to 8.1 mg/kg/hr iv[61].

Antisecretory Thioamides - Since the identification of 2-phenyl-2-(2-pyridyl)-thioacetamide (SC 15396)[90] as a non-anticholinergic antisecretory compound, which was effective in several species and capable of suppressing both histamine and gastrin induced secretion, reports have issued on structure-activity relationships among thiocarboxamides. In one study[63] on 22 compounds within this class, antisecretory activity seemed related to the NCCC(=S)N grouping. The nitrogen in the Y position was necessary for activity, and it could be a constituent in a ring. The influence of structure on antigastrin, antisecretory, and antiulcer activities were studied in several compounds related to SC 15396[64]. For antigastrin activity and perhaps antisecretory as well, the critical factor appeared to be the distance between the N and S, a finding that compared favorably with the previous study. The most potent antisecretory agent emerging from these studies was 2-pyridyl-thioacetamide.



2-pyridyl thioacetamide

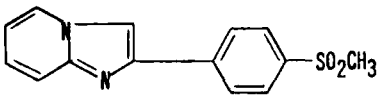
This compound has been shown to inhibit basal gastric acid secretion, as well as secretion provoked by challenges of gastrin, pentagastrin, histamine, and 2-deoxy-D-glucose[65]. It also has been shown to protect against experimentally induced gastrointestinal erosions in rats, cats, and guinea pigs. The mechanism of action is not clear, although some evidence suggests an antigastrin action[64]. In addition to suppressing pentagastrin induced secretion, 2-pyridyl-thioacetamide inhibits the effect of pentagastrin on blood vessels and hamster fundic strip[66].

Licorice Derivatives - Carbenoxolone sodium, the di- sodium salt of 3-O (β-carboxyproprionyl)-11-oxo-18β-olean-12-en-30-oic acid, has undergone several therapeutic trials in gastric ulcer patients over the past two years[67]. With only one exception among eight different trials, carbenoxolone was shown to either heal gastric ulcers or significantly decrease the size of the ulcer. Trials in duodenal ulcer patients with a special formulation to aid absorption from the duodenum have suggested efficacy, but the data are equivocal; the results seem to depend on the patient population, method of assessing improvement, and whether the individuals are ambulant[67-69]. In a recent double-blind study healing seemed enhanced early in treatment, but on longer term no difference from placebo was observed[69]. Carbenoxolone has been shown capable of altering mucus secretion[70], prolonging epithelial life by decreasing turnover rate, and inhibiting pepsin[71], in addition to facilitating opposition of the gastric mucosa to the destructive properties of bile[72].

Side effects encountered with carbenoxolone (hypertension, fluid retention, and hypokalemia) led to clinical trials with other licorice derivatives. Early studies indicated effectiveness of deglycyrrhizinated licorice in gastric ulcer, but additional information on its efficacy is needed - especially in view of the large multi-center study[73] and another[74] which did not show any advantage with the active treatment when compared to

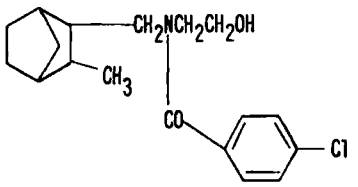
placebo. Animal studies with lauroyl glycyrrhetinic acid (BX 24) suggested efficacy in gastric ulcer which subsequently was not proven when tested in man[75].

Zolimidine - This compound first disclosed in 1965, has been shown to reduce the incidence of stress ulceration in rats presumably by producing quantitative and qualitative changes in gastric mucus. Subsequent clinical trials showed that daily doses of 800-1200 mg increased the mucoprotein content of gastric juice in patients with gastric and duodenal ulcers, but had little effect in patients with gastritis[76].



Zolimidine

Chlorocanfamide - This compound, whose synthesis was recently published, is one of a large series of spasmolytic compounds with local anesthetic activity[77]. In the anesthetized gastric lumen-perfused rat, it has been shown to inhibit secretion stimulated by histamine but not that stimulated by carbachol; its anticholinergic activity in the guinea pig ileum is weak compared to atropine. Previously published clinical trials showed this drug capable of reducing basal gastric acid output as well as that stimulated by histamine, pentagastrin, and food after daily treatment with 500 mg for 12 days[78].



Chlorocanfamide

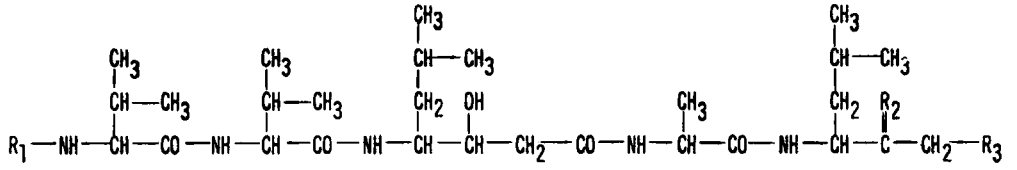
Other Structures - In light of reports that diazepam inhibits basal secretion in rats and humans[79], the finding of antisecretory activity among some analogs was of interest. These compounds, which lacked the imino N in the lactam ring and whose CNS activities did not resemble those of diazepam, nevertheless inhibited basal secretion in rats at doses of 30-50 mg/kg po[80].

Several compounds in a series of p-aminobenzamidopyridines effectively inhibited stress ulceration in rats at oral doses of 35-40 mg/kg; some of these also had mild CNS depressant activity[81]. In a large series of pyridines, significant antisecretory activity was found for 3- and 4-phenoxypyridine, 2-phenylpyridine N-oxide, 2-(2-thienyl) pyridine, 3-phenylpyridine, and 2,2-bipyridine[82]. Clinical trials were reportedly planned for the latter compound.

Inhibitors of Pepsin Activity - The literature on pepsinogens, pepsins, and pepsin inhibitors has been reviewed by Samloff[83]. A new glycopeptide, isolated from hog duodenum and synthetically sulfated (GLPS), has been described as having anti-inflammatory, antiulcer, and antipeptic activity. As with the sulfated polysaccharides (carrageenan, amylopectin sulfate[84], etc.), GLPS seems to exert its antipeptic action by complexing with substrate; an oral dose of 200 mg completely inhibited peptic activity in 2 of 4 patients for up to 1 hour. GLPS is apparently not absorbed or degraded

by gut microflora. (For bibliography, see reference 85.)

New types of pepsin inhibitors have been reported by Japanese investigators who isolated them from culture filtrates of various species of actinomycetes, particularly Streptomyces. These small peptide substances, very similar in structure, are marked by the presence of an unusual naturally-occurring amino acid, 4-amino-3-hydroxy-6-methylheptanoic acid[86,87].



		R_1	R_2	R_3
I	Pepstatin A	iso-valeryl	<H OH	-COOH
II	Pepstatin B	n-caproyl	<H OH	-COOH
III	Pepstatin C	iso-caproyl	<H OH	-COOH
IV	Pepstanone	iso-valeryl	=O	-H
V	S-PI	acetyl	<H OH	-COOH

Pepstatin A has been reported to be specific against acid proteases, to have little toxicity, and to protect against ulcers induced by pyloric ligation in rats[88]. An oral dose of 50 mg inhibited peptic activity in human gastric juice by 90-100% for at least 1 hour[88]. Both Pepstatin A and S-PI act by selectively binding to the enzyme and not by binding substrate, as do the sulfated polysaccharides and GLPS[89,90]. Pepstatin A has also been reported to inhibit renin and sustained pressor principle[91,92].

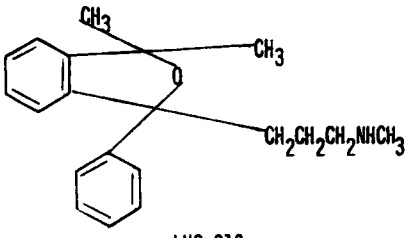
Catecholamines and Gastric Secretion

In dogs isoproterenol inhibited feeding and pentagastrin induced secretion, but had either no effect or an augmented one on histamine mediated secretion[93,94]. Other β agonists, terbutaline and salbutamol, also inhibited pentagastrin induced gastric secretion in a dose related manner. Measurement of the ratio of mucosal blood flow to secretory volumes suggested that the inhibitory effect of isoproterenol was not due to alterations in mucosal blood flow. Interestingly, propranolol did not antagonize isoproterenol inhibition whereas it did the other β agonists[95].

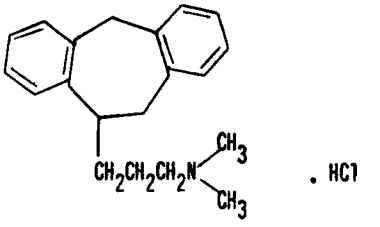
The β agonist, nylidrin (Arlidin, USV), was found to produce dose related increases in gastric acid secretion in pigeons and man[96,97]. Propranolol antagonized these increases and by itself was found to inhibit histamine induced secretion in man[97]. Other studies in the dog[98] and man[99] showed that propranolol augmented steady state pentagastrin induced secretion and increased basal secretion, respectively. In contrast, recent studies in rats showed that propranolol significantly diminished gastric acid secretion and formation of gastric ulcers[100]. From these studies it is difficult to predict the effect of β agonists or antagonists on gastric acid secretion. It is hoped that future studies in man will both ascertain

their effects and aid in the selection of experimental animal preparations that will be predictive.

Certain compounds that block catecholamine uptake also are antisecretory and antiulcer; imipramine and DMI are examples[1]. LU3-010 inhibited basal, pentagastrin, reserpine, and histamine induced gastric secretion and reserpine ulceration in the rat. This compound was shown to have the



LU3-010



AY-8794

lowest ED_{50} for secretory inhibition of a series of 9 compounds that were tested[101]. In further studies to determine the relationship between catecholamine uptake and secretory inhibition, AY-8794 was compared with butriptyline, imipramine, and trimipramine[102]. AY-8794, the analog containing an unbranched aliphatic side chain, inhibited uptake of labeled norepinephrine in the mouse and rat heart, and gastric secretion in the rat. Butriptyline was less effective in both respects. These data, in addition to studies in prior literature, continue to suggest a correlation between blockade of catecholamine uptake and inhibition of gastric secretion.

Agents Affecting Gastrointestinal Motility

We have not de-emphasized this section without some deliberation; there is a paucity of information on new compounds which affect motility. Even in the area of prostaglandins, whose stimulation of motility is well-known and whose antiulcer activity is being feverishly exploited, there seems to be no effort to develop from this class a safe and clinically useful laxative. Possibly Burnstock's summary of evidence for a new neuromuscular effector system - purinergic nerves - will serve to stimulate new attacks on the control of gastrointestinal motility from the chemical and pharmacological disciplines[103].

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Section III - Chemotherapeutic Agents

Editor: George H. Warren, Wyeth Laboratories, Inc., Philadelphia, Pa.

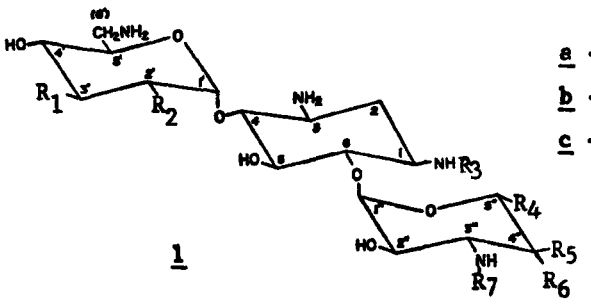
Chapter 12. Antibiotics

K. E. Price and F. Leitner, Bristol Laboratories, Syracuse, N. Y. 13201

General - Reviews on the mode of action[1], biology[2], and clinical use[3] of antibiotics were published. The rifamycins[4,5], coumermycins[6], chloramphenicol[7], cephalexin[8], epicillin, minocycline, and spectinomycin[9] were reviewed. A monograph appeared on the chemistry and biology of β-lactam antibiotics.[10] The proceedings of the First International Symposium on Infectious Antibiotic Resistance were published.[11] A general discussion on mechanisms of bacterial resistance to antibiotics[12] and on the properties of episomes mediating drug-resistance[13] appeared.

Infectious resistance to antibiotics - An R factor that confers resistance to chloramphenicol by decreasing the permeability of the cell membrane to the drug was isolated from Escherichia coli strains of clinical origin.[14] Instances of presumed interbacterial transfer of R factors in mammalian hosts were reported.[15-17] Requinomycin, a new antibiotic that prevents the transfer of R factors between E. coli strains, was described.[18,19]

Aminoglycosides - In vitro data comparing the antibacterial potency of tobramycin (1a) and gentamicin continue to flood the literature with the majority of papers indicating, as do these representative publications[20,21], that the former is 2- to 4-fold more active than the latter against Pseudomonas aeruginosa, but significantly less effective against Serratia marcescens. The response of most other microorganisms to these antibiotics is virtually identical. A question still to be resolved is that raised by



1

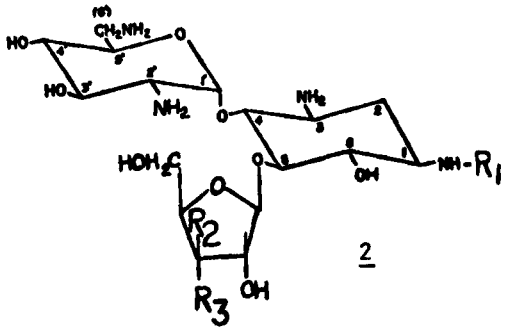
	R_1	R_2	R_3	R_4	R_5	R_6	R_7
a	-H	$-NH_2$	-H	$-CH_2OH$	-OH	-H	-H
b	-OH	-OH	-H	-H	$-CH_3$	-OH	$-CH_3$
c	-OH	-OH	$-C(=O)-CHOH-CH_2-CH_2-NH_2$	$-CH_2OH$	-OH	-H	-H

conflicting reports about their degree of cross-resistance. Although all members of a series of Pseudomonas sp.[22] and Proteus rettgeri strains[23] isolated from single hospital sources were reported to have resistance to both tobramycin and gentamicin, other investigators[24,25] have not confirmed

this observation. In guinea pigs the cochlear toxicity of tobramycin was slightly less than that of gentamicin.[26] Peak serum concentrations and half-lives of the antibiotics are generally similar in humans, both in normal volunteers[27,28] and in anephric subjects.[29] Initial clinical studies with tobramycin show it to be effective in the treatment of urinary tract infections[30] and non-bacteremic Pseudomonas infections.[31]

A new enzymic assay for monitoring gentamicin serum levels based upon the inactivation of the antibiotic through 2"-O-adenylylation has been described.[32,33]

The structure and biological activity of Sch. 14342 (1b), a previously uncharacterized component of the gentamicin fermentation, has been reported.[34,35] This antibiotic, now designated gentamicin B, has an antibacterial spectrum similar to that of gentamicin, but has only about one-third its activity and acute toxicity. However, it is claimed to possess relatively low chronic oto- and nephrotoxicity. The biological properties of several other new naturally-occurring pentose-containing aminoglycosides have now been reported. Butirosins A (2a) and B (2b)[36-38], ribostamycin (2c)[39], and lividomycins A[40] and B[41] all display excellent activity against Enterobacteriacese and Staphylococcus sp. In addition, all but ribostamycin are moderately inhibitory for P. aeruginosa strains. Although



2

	R_1	R_2	R_3
a	$-C(=O)-CH(OH)-CH_2-CH_2-CH(NH_2)-CH_2-NH_2$	-OH	-H
b	$-C(=O)-CH(OH)-CH_2-CH_2-CH(NH_2)-CH_2-NH_2$	-H	-OH
c	-H	-H	-OH

the butirosins are active against many organisms that inactivate kanamycin by virtue of 3'-O-phosphorylation, a newly described 3'-phosphotransferase has been identified that is capable of inactivating both kanamycins and butirosins.[42,43] Lividomycins, which lack a 3'-hydroxyl group, are nevertheless inactive against bacterial strains that produce an enzyme that phosphorylates their 5"-hydroxyl group.[44,45] Chemical procedures describing the synthesis of 6-N-methylkanamycins[46], 3'- and 4'-O-methylneamines[47], 3',4'-deoxy- and 3',4',5"-trideoxyribostamycins[39], lividomycin A 5"-phosphate[48], 5"-deoxylividomycin A[49] and B[41], 5"-aminolividomycin A[49], as well as the conversion of lividomycin A into B[50], and ribostamycin into butirosin B[51] have been reported. In addition, glycosylation procedures have been utilized to prepare ribostamycin[52], 3'-deoxykanamycin A[53], 5-glucosylneamine[54], paromamine[55], and an uncharacterized glucose derivative of kanamycin A.[56]

The antibacterial spectrum of butirosin B was significantly broader

than that of ribostamycin, its 1-amino analogue. A similarly acylated derivative of kanamycin A, 1-N-γ-amino-α-hydroxybutyryl kanamycin A, was prepared and given the designation BB-K 8 (1c).[57] Subsequent studies have shown that its oto- and nephrotoxic potential[58] as well as its pharmacokinetic characteristics[59] are similar to those of kanamycin A. BB-K 8 has the broadest antibacterial spectrum of all the aminoglycosides, principally because it is a poor substrate for the majority of known bacterial enzymes that inactivate this class of antibiotics.[60]

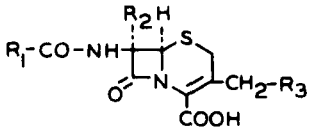
Clinical trials continue to show that spectinomycin hydrochloride administered as a single 2- or 4-g dose to men or as a single or double treatment at these doses to women produces cure rates in gonorrhea that regularly exceed 90%.[61,62] The stereochemistry and absolute configuration of the antibiotic have now been established by X-ray diffraction studies.[63]

The relative affinities for binding sites on 70S E. coli ribosomes of 10 streptomycin derivatives correlate with their antibacterial activity. High affinity was observed only with derivatives which have intact streptidine and a methylaminostreptose moiety.[64] Kasugamycin was prepared by total synthesis[65] and the structure of validamycin A, an aminocyclitol antibiotic that is also used for control of rice plant diseases, was elucidated.[66]

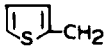
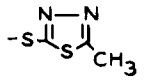
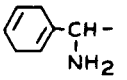
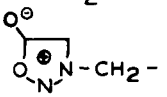
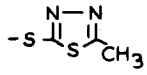
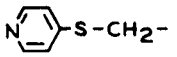
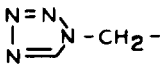
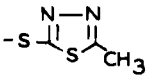
β-Lactams - The discovery of cephamycins stimulated interest in 7(6)-substituted β-lactam antibiotics. Cefoxitin (3a), a semisynthetic cephamycin, though less active than cephalothin and cephaloridine against gram-positive organisms, has a broader spectrum against gram-negative bacteria.[67,68] Numerous 6α-substituted penicillins and 7α-substituted cephalosporins have been synthesized but, from the scanty biological data available, it would appear that, 7α-methoxycephalosporins excepted, this type of substitution decreased antibiotic activity.[69-74]

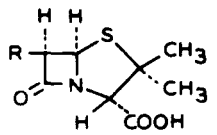
Penam derivatives of a novel type, the 6β-amidinopenicillanic acids (4a), were described.[75] These compounds, typified by 4b (FL1060), are exceptionally active against most gram-negative pathogens but less active than conventional penicillins against Neisseria, Haemophilus, and gram-positive species. Cells of E. coli exposed to 4b become spherical but are osmotically insensitive and lyse without forming spheroplasts. For this reason, it was suggested that the mode of action of 4b differs from that of the true penicillins.

An oxygen analogue of penicillin V (4c) had little or no activity against a variety of bacteria.[76] Two closely related procedures for the conversion of natural penicillins to ampicillin, without involving 6-APA as an intermediate, were patented.[77,78] Acetyl mixed anhydrides were used to block carboxylic acids during 6(7)-side chain cleavage of penicillin G and cephalosporin C.[79] Cephalexin, cephaloglycin, and other cephalosporins were synthesized by the enzymic condensation of α-amino acid esters with 7-ADCA or 7-ACA.[80] Whole cells of Xanthomonas and other Pseudomonadaceae mediate the reaction with yields of up to 90%.



3

	R_1	R_2	R_3
a.		$-OCH_3$	$-O-CONH_2$
b.	$C_6H_5-CH_2-\underset{\underset{NH}{\|}}{C}-NH-CH_2-$	$-H$	
c.	D 	$-H$	$-H$
d.	$NC-CH_2-$	$-H$	$-O-CO-CH_3$
e.		$-H$	
f.		$-H$	$-O-CO-CH_3$
g.		$-H$	



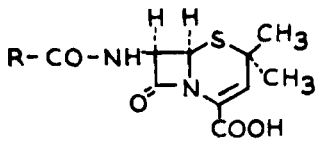
4

	R
a.	$R_1R_2N-CH=N-$
b.	
c.	$C_6H_5-O-CH_2-CO-O-$
d.	D p-$HO-C_6H_5-CH(NH_2)-CO-NH-$
e.	D $C_6H_5-CH(N_3)-CO-NH-$
f.	D $C_6H_5-CH(SO_3H)-CO-NH-$

Procedures for the synthesis of 2α-alkoxy-[81], 3-alkoxymethyl-[82], 4-carboxymethyl-[83], and 3-unsubstituted[84] cephalosporins were developed. The cephalocillins (5), compounds that share structural characteristics with penicillins as well as cephalosporins, were synthesized.[85] The reduction of cephalosporins with chromium(II) salts[86] or by an electrochemical procedure[87] led to the formation of 3-methylene-cephams (6), which in turn can be converted quantitatively to 3-methylceph-3-ems by intramolecular rearrangement. Cephalexin can thus be obtained from 7-ACA with 6a as intermediate.[87]

The first conversion of a cephalosporin to a penam was reported independently from 2 laboratories.[88,89] Novel conversions of penicillins into cephalosporins were also described.[90,91] A key intermediate (7) in the total synthesis of cephalosporins (according to the method of Woodward) was prepared from a penicillin.[92] Compound 7 should be a useful starting material for the synthesis of nuclear analogues of penicillins and cephalosporins. A homoceph-4-em (8) prepared from 7 lacked antibacterial activity.[93] Nuclear analogues of cephalosporins (9,10) were also obtained by total synthesis involving β-lactam ring closure by the photolysis of diazo-

malonic ester amides.[94,95] Compounds 9 and 10 had no antibacterial activity. Disulfide analogues of a penicillin (11,12) were prepared[96] and stereochemically defined.[97] They were less active than the parent penicillin.



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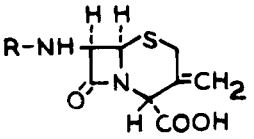
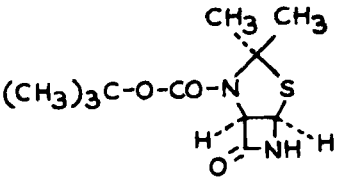
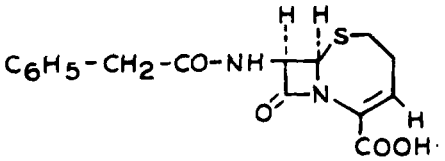


a R= H

6



7



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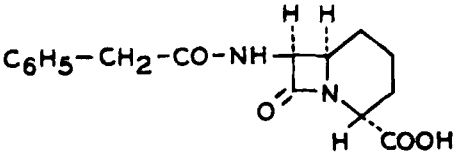

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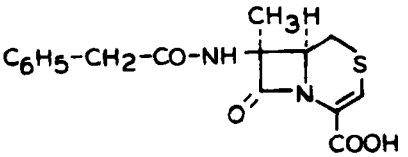
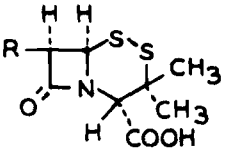


10



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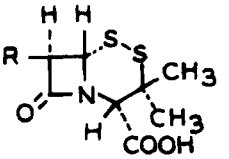


12

Laboratory data on a new broad-spectrum cephalosporin, BL-S 339 (3b), were published[98] and activity, as a function of structure, among a group of compounds related to the former, was discussed.[99] Several cephalosporins are at various stages of clinical investigation. Cephradine (3c), chemically related to cephalexin and similar in activity, was effective, orally and intramuscularly, in urinary tract infections[100], and orally, in infectious enteritis caused by Salmonella and Shigella[101] (82% overall cure rates). In patients with disseminated cancer suffering from urinary, broncho-pulmonary, and wound infections, orally-administered cephradine was effective in 63% of the cases.[102] Cephacetrile (3d) has an antibacterial spectrum similar to that of cephalothin.[103] In man, peak serum concentrations ranged from 20-35 μg/ml about 1 hr after im admini-

stration of 1 g.[103] In normal subjects, cephacetrile is excreted by glomerular filtration and tubular secretion.[104] After iv administration of 500 mg, 84% of the dose was recovered in the urine.[105] Cephacetrile was effective clinically.[103] Cephanone (3e) has a broader antibacterial spectrum than cephalothin for it is active against some strains of Enterobacter.[106] After im administration of 1 g to man, cephanone reached peak serum concentrations of 57 μg/ml and had a serum half-life of 174 min.[107] Recent clinical results showing good efficacy and tolerance with cephapirin (3f)[108-111] were in agreement with previously reviewed data. Cefazolin (3g) gave cure rates of 80-86% in the treatment of infections caused by a variety of pathogens.[112,113] Side effects were minimal.

A number of penicillins also reached the stage of clinical trials. In the oral treatment of common infections, amoxycillin (4d) given t.i.d. was as effective as similar individual doses of ampicillin administered q.i.d.[114] Pivampicillin was effective in the treatment of respiratory and urinary tract infections caused by ampicillin-sensitive organisms.[115] Two cases of enterococcal septicemia were successfully treated with azidocillin (4e).[116] This compound was also the subject of an extensive pharmacokinetic study in laboratory animals.[117] A similar investigation comparing sulfocillin (sulbenicillin) (4f) with carbenicillin was done.[118]

β-Lactamase inhibitors - Several reports have described naturally-produced substances that antagonize the action of β-lactamases and thus prevent degradation of hydrolyzable penicillins and cephalosporins. Two such compounds are apparently macromolecules[119,120] while 2 others are dialysable and are presumed to have molecular weights in the order of 400.[121] Another series of low molecular weight azetidinones have similar activity.[122]

Tetracyclines - New derivatives active against tetracycline-resistant bacteria include several where tetracycline's 2-carboxamido group has been modified to give N-alkyl[123] or -alkenyl[124] preparations and acylated 5a,6-anhydrotetracyclines.[125]

Doxycycline was effective when used by the iv route in hospitalized patients with a variety of severe maladies including septicemia, soft tissue, and urinary tract infections.[126,127] Orally-administered doxycycline proved to be effective when given as a single 300-mg dose in treatment of gonorrhea and non-specific urethritis in men[128] and gonorrhea in women.[129]

Minocycline is almost completely absorbed upon oral administration, but is apparently metabolized to some extent since all absorbed drug cannot be accounted for.[130] In vitro tests indicate that the compound is not only markedly inhibitory for most staphylococcal strains, but for many gram-negative species of bacteria.[131] Preliminary clinical studies show the compound to be effective in treatment of various venereal diseases, including primary and secondary syphilis.[132] Minocycline was moderately effective in eliminating the meningococcal carrier state in 2 groups of military trainees.[133,134]

Macrolides - Macrolides having a 16-membered lactone ring were classified

into 3 groups on the basis of the number and position of their carbonyl groups. The contribution of these functions to antimicrobial activity was considered.[135] A general synthetic method for macrocyclic lactones involving cyclization of allylic dibromides by nickel carbonyl was described.[136] A new macrolide, rosamicin, initially named rosaramicin, was isolated from fermentation broths of Micromonospora rosaria.[137] Structural studies have revealed that it is similar to cirramycin A_1, the aglycone ring being the same, but differing in that it has a desosamine moiety corresponding to cirramycin's mycaminose.[138] Its antimicrobial activity, particularly against gram-negative bacteria, may be greater than that of erythromycin.[139] Conformational analysis of the leucomycins was completed[140] and a laboratory study with one of them, leucomycin A_3 (josamycin) showed that about 70% of randomly selected staphylococcal strains were susceptible to it.[141] However, pharmacokinetic studies in man indicate that josamycin is not well absorbed after oral administration.[142] Allylic rearrangement of the 16-membered macrolides, SF837 and SF837 A_2, gives antibiotics with lower toxicity and better antibacterial activity.[143] Virginiamycin, a mixture of antibiotics, has been shown to be composed of a macrocyclic lactone that is identical to streptogramin A (mikamycin A) and a depsepeptide.[144] The proposed structure of angolamycin (shincomycin A) shows it to have the same molecular formula as tylosin but differs in that it has a deoxymycaminose and an epoxy bridge (C-12,13) in the aglycone ring.[145]

Ansamycins - It has been suggested that the problem of resistance development to rifampicin could be reduced by using it in combination with other antimicrobials since many such mixtures act synergistically in the therapy of experimental animal infections.[146]

In contrast to dapsone, rifampicin readily kills Mycobacterium leprae present in the tissues of experimentally infected mice.[147] The rapid bactericidal effect of rifampicin, relative to that of dapsone, observed in mice was also found to occur in the lesions of lepromatous leprosy in man.[148]

Tolypomycin Y is a new ansamycin produced by Streptomyces tolypophorus. It is found concurrently in fermentation broths with rifamycins B and O.[149] It is cross-resistant with rifamycins but not other antibiotics and has antibacterial activity against gram-positive organisms that is comparable to that of rifampicin. Its gram-negative inhibitory effects, however, are 2- to 4-fold less than those of rifampicin. Tolypomycin Y can be differentiated from the rifamycins on the basis of its superior activity against Streptomyces alcalophilus.[150] Its structure has not yet been determined.

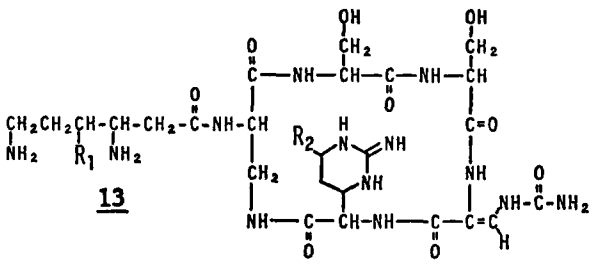
Lincomycin - Clinical reports depicting efficacy of oral clindamycin therapy continue to mount. A high degree of therapeutic success has been reported in cases of streptococcal pharyngitis[151], acne vulgaris[152], Mycoplasma infection[153], otitis media[154], and infections caused by various anaerobic bacteria.[155-158] Pharmacokinetic studies in man with orally-administered clindamycin HCl-monohydrate reveal that an equilibrium state is reached after the 4th or 5th dose, that the drug is not accumulated,

and that it does not stimulate its own metabolism.[159] Relatively high concentrations of clindamycin can be achieved in bone following standard dosage (300 mg) of the drug.[160] Clindamycin-2-phosphate, a new parenteral dosage form of clindamycin, has reduced irritation liability relative to the parent compound. The esterified form is inactive *per se* but is rapidly hydrolyzed *in vivo* to clindamycin.[161] Initial clinical studies indicate that the compound is well-tolerated and highly effective in infections caused by various facultative gram-positive[162-165] and anaerobic[166] bacteria.

New celesticetins, members of a class of antibiotics which are related to the lincomycins, have been produced by chemically-derived mutants of *Streptomyces caelestis*. None of the N-demethyl and/or 7-O-demethyl celesticetins isolated were as active as celesticetin itself.[167,168]

Peptides - Jolipeptin and gatavalin are 2 new heat-labile polypeptide antibiotics produced by a colistin-producing strain of *Bacillus polymyxa*.[169,170] The former acts on the cell membrane of both gram-negative and gram-positive bacteria, while the latter is inhibitory for gram-positive bacteria only.[171] Thiopeptin, a sulfur-containing antibiotic is comprised of a major component (thiopeptin B) and 4 minor ones (A_1 to A_4).[172] All have similar biological properties displaying activity against both gram-positive bacteria and *Mycoplasma*. The complex reportedly acts as a growth promotant when incorporated into animal feeds.

Two minor components isolated from fermentations of *Streptomyces griseoverticillatus* var. *tuberacticus*, the tuberactinomycin N (13a)-producing organism, have been designated tuberactinomycins B (13b) and O (13c).[173] The structural relationships of these antibiotics and a 4th congener in this group, tuberactinomycin A (13d), have now been established with the recognition that tuberactinomycin B is identical to the known antimycobacterial antibiotic, viomycin.[174] Preparation of chemically modi-



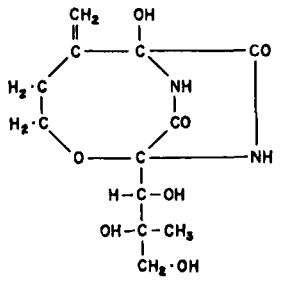
13

	R_1	R_2
a	-OH	-H
b	-H	-OH
c	-H	-H
d	-OH	-OH

fied derivatives of viomycin has permitted the identification of the reactive functions that are critical for antibacterial activity.[175] The total synthesis of negamycin and its antipode has been accomplished.[176] The miscoding capability of the former resembles that of streptomycin and kanamycin, although ribosomes obtained from bacterial strains resistant to these antibiotics retain almost complete susceptibility to the antagonistic effects of negamycin.[177] A new peptide antibiotic, fumarylcarboxamido-L-2,3-diaminopropionyl-L-alanine, active against gram-negative bacteria, was isolated from fermentation broths of *Streptomyces collinus*.[178] A

cyclic structure has been proposed for epidermidin A_1, one of a series of 4 related antibiotics from Staphylococcus epidermidis. Its tentative amino acid sequence is: cyclo-lys-ala-asp-glu-ser-leu-thr-gly-val-gly-arg.[179]

Miscellaneous - Bicyclomycin (14), an antibiotic produced by Streptomyces sapporonensis, is moderately active against gram-negative pathogens (excluding Pseudomonas and Proteus) and inactive against gram-positive organisms.[180] Per os, the compound is not well absorbed, but derivatives obtained by esterification of the primary alcohol are.[181] These esters, generally inactive, are converted to the parent compound in vivo. Bicyclomycin was effective in the treatment of mice infected with E. coli.[180] In man, serum concentrations reached a peak of 32 μg/ml 1 hr after im administration of 1 g, and 95% of the administered dose was recovered in the urine.[182] The chemical structures of 3 antibiotics, albofungin[183], ikarugamycin[184-186], and thermorubin A[187] were elucidated.



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Chapter 13. Antifungal Agents

J. Allan Waitz and C. G. Drube
Schering Corporation, Bloomfield, New Jersey

Reviews - A review of pharmacokinetics of antifungal therapy includes data on several systemic antifungals[1]. Griseofulvin and clotrimazole are reviewed relative to their mechanism of action[2]. Although amphotericin B, pimaricin and nystatin are included in the treatment of fungal infections in otorhinolaryngology, emphasis is on antiinfective mixtures[3]. Two reviews cover chemotherapy of mycoses, including most clinically useful antifungal agents,[4] and hamycin, 5-fluorocytosine and clotrimazole[5]. Experimental dermatophyte infections in man are reviewed but therapeutic discussions are limited to griseofulvin and sodium omadine[6]. A review related to problems in therapy and diagnosis of systemic candidiasis covers therapeutic evaluation of amphotericin B, 5-fluorocytosine, and clotrimazole[7].

Methods - Intraperitoneal inoculation of cynomolgus monkeys with Histoplasma capsulatum produces a mild form of histoplasmosis[8]. In experimental urinary tract infections of mice with Candida albicans, pre-inoculation gonadectomy of both male and females increased resistance to the infection[9]. Experimental coccidioidomycosis in mice has been described in detail[10]. Experimental gastrointestinal moniliasis in 3-5 day old quail produced by oral inoculation of C. albicans in drinking water has been used for evaluation of ethylenediamine dihydro iodide, nystatin, sodium propionate, gentian violet and benlate (methyl 1-(butyl-carbamoyl)-2-benzimidazole carbamate)[11]. Descriptions of dermatomycoses in chinchilla, rabbits, ferrets and mink[12] may lead to development of further experimental topical infections.

Need for improved methods of isolation of pathogenic fungi has led to development of paper impregnated with cycloheximide and chloramphenicol useful in conjunction with agar media[13]. The selective indicator medium, DTM introduced in 1969, has been favorably appraised[14]. A defined synthetic amino-acid medium for fungi (SAAMF) is useful for in vitro susceptibility testing, in particular with 5-flurocytosine and clotrimazole[15]. New methods have been developed for growth of T. mentagrophytes on fabric,[16] and for evaluation of skin permeability using rabbit, pig and human skin[17]. In vitro culture of dermatophytes on isolated stratum corneum is useful for study of topical antifungal agents, in particular haloprogin[18].

For stabilization of griseofulvin suspensions, polyvinyl

alcohol was superior to polyvinyl pyrrolidone, Tween 80, sodium carboxy methyl cellulose, kaolin and dry milk[19]. A useful method for evaluation of permeation of antimycotic agents in the skin based on microscopic examination of stripped stratum corneum in polarized light has been developed[20]. Details of assay methods have been provided for amphotericin B and nystatin,[21] cycloheximide,[22] griseofulvin,[23] pyrrolnitrin,[24] and tolnaftate[25]. A simple agar diffusion microbiological assay method for serum levels of 5-fluorocytosine uses Saccharomyces cerevisiae[26]. Gas chromatographic assay of griseofulvin is useful for substance and dosage forms; dechlorogriseofulvin can be determined simultaneously[27]. Quantitative determinations of griseofulvin in skin, plasma and sweat utilizes ether extraction and spectrophotofluorometric analysis or gas liquid chromatography[28]. Methods have been presented for isolation of actinomycetes and other microorganisms from marine environments. A large proportion of the isolates studied produced antifungal activity[29,30].

Clinical Experience - Intravenous administration of amphotericin B for systemic mycoses; oral therapy with griseofulvin for a number of dermatophyte infections, topical nystatin for Candida and topical tolnaftate for dermatophytes, continue to be the main-stays in therapy of fungal diseases. Side effects of intravenous amphotericin B therapy have been evaluated further; fever, chills, weakness, nausea, and nephrotoxicity were the prime consistent toxic manifestations[31]. In dogs, simultaneous administration of intravenous mannitol to improve renal blood flow prevented the marked rise in BUN and serum creatinine values, as well as vacuolation of renal tubular epithelium observed with amphotericin B alone[32].

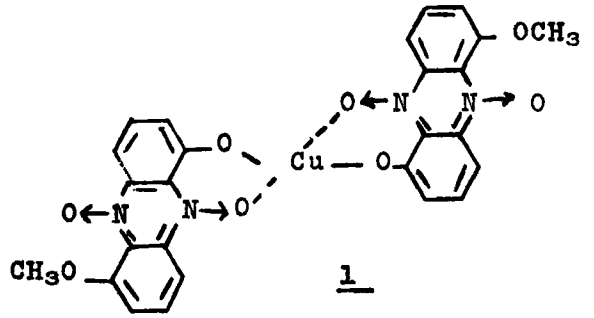
The histoplasmosis cooperative study has given dosage recommendations for amphotericin B for chronic pulmonary histoplasmosis based on studies in 85 patients[33]. Among many clinical mentions of griseofulvin, two worthy of mention are: the finding of significant levels of griseofulvin in the stratum corneum indicating that it can act as a compartment for griseofulvin;[34] a report of nearly 50,000 cases of tinea capitis in Israel, 38,000 of which were treated with standard dose griseofulvin[35].

Additional reports relate to the increasing use of oral 5-fluorocytosine for systemic candidiasis and other systemic mycoses[36,37,38]. Oral administration of 5-fluorocytosine results in rapid appearance of high serum and CSF levels with peaks related to degree of renal function[39].

Oral therapy of chronic mucocutaneous candidiasis with clotrimazole resulted in decrease in clinical symptoms although relapses were common[40]. Oral clotrimazole was not

effective for coccidioidomycosis[41]. Topical application of clotrimazole is promising on the basis of *in vitro* and experimental animal models[42] and good tolerance in man[43]. In 54 patients treated topically, most showed improvement after 1-2 weeks of therapy[44]. In 54 patients treated topically, most showed improvement after 1-2 weeks of therapy[44]. After long-term observation of 95 patients with mycoses treated topically with clotrimazole, 60% were healed and 34% showed improvement; local tolerance was good[45]. Topical pimaricin has been effectively used in therapy of *Fusarium* corneal ulcers[46]. Among the clinical reports appearing on tolnaftate, is the first apparent case of allergic contact delayed hypersensitivity to tolnaftate[47]. Topical miconazole is useful in the therapy of vaginal candidiasis,[48,49] as well as other topical *Candida* and dermatophyte infections[50]. The effectiveness of haloprogin in several clinical studies has been reported[51-54].

New Antifungal Agents - A series of 5,7-difluoro, dichloro, dibromo and diiodo quinolines and 8 amino quinolines have been prepared and tested for antifungal activity. The 5,7-difluoro derivative of quinoline was most active, while other halo derivatives were less so. Eight nitro quinolines showed greater activity than quinoline but halo substituted 8 nitro quinolines other than fluoro were less active. Amongst 8-aminoquinolines, all halo analogues were less active than the parent compound[55]. Of many 7 and 5,7 substituted 8 quinolinols, the most active were the 7-bromo, 7-iodo,7-chloro-5-fluoro, 7-bromo-5-fluoro, 5-chloro-7-fluoro, and 5-bromo-7-chloro derivatives[56]. In a study of 5,7 substituted 2-methyl-8-quinolinols, the 5,7-dichloro and 5,7 dibromo were the most active. With the exception of these 2 compounds, and 5-iodo-2-methyl-8-quinolinol, the 2-methyl analogues were less active than the corresponding 8-quinolinols[57]. The cupric complex of 6-methoxy-1 phenazinol 5,10 dioxide (copper myxin 1) showed good topical activity against bacteria, yeasts and



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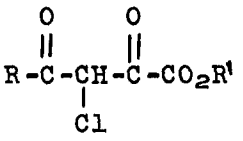
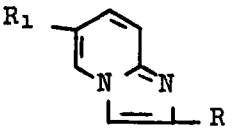
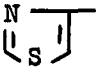
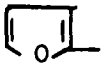
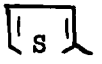
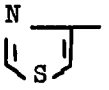
dermatophyte infections in experimental animals[58]. Of analogues of acylpyruvates showing activity against *C. albicans* and *Microsporum canis*, 2-8 were highly active, although acting as strong vesicants. 8 was also active at low levels against



	R	R'
2	CH_3	CH_3
3	CH_3	C_2H_5
4	CH_3	$i\text{-}C_3H_7$
5	CH_3	$n\text{-}C_4H_9$
6	C_2H_5	C_2H_5
7	$i\text{-}C_4H_9$	C_2H_5
8	2-Naphthyl	C_2H_5

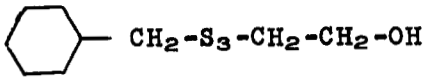
other Candida species, Torulopsis and Aspergillus niger[59]. In a continuing study of activity of organic sulfur compounds against Histoplasma, 61 compounds representing 13 classes of organic compounds were studied. Significant activity was seen amongst certain thiols related to p-chlorobenzenethiol, diacetyl sulfide and diacetyl disulfide, certain thiosulfonates, and simple thiol and thion esters[60]. Of over 50 imidazo (1,2-a) pyridine compounds evaluated, 9-13 were the most



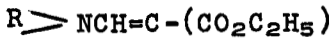
	R	R_1
9		H
10		H
11		H
12	CH_3OCONH	H
13		CH_3

active as antifungals[61]. Cinnamylpyrogallol was found to be a better inhibitor of fungal growth than cinnamyl alcohol, pyrogallol, or cinnamylphloroglucinol[62]. Glutaraldehyde also demonstrated broad-spectrum antifungal activity[63]. Of a

series of substituted nitrobenzenes and anilines, 1,3-dichloro-5-nitrobenzene, 1,3-dibromo-5-dibromo-aniline showed useful levels of activity[64]. 14 is the active antifungal

14 

substance from Petiveria alliacea and has been synthesized[65]. Amongst a series of fused pyrimidines, 15-18 were active



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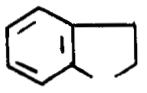

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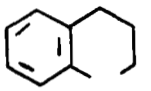

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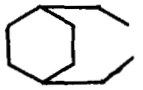

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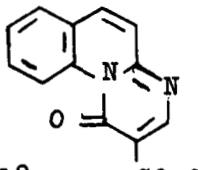


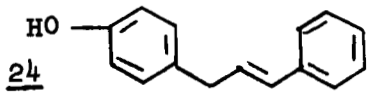
18 $CO_2C_2H_5$

against T. mentagrophytes at high levels[66]. In a series of aliphatic amines, 19-23 were most active against both

$$RNH(CH_2)_2CO_2Et$$

	R
19	$C_{10}H_{21}$
20	$C_{12}H_{25}$
21	$C_{11}H_{23}$
22	$C_{15}H_{31}$
23	$C_{16}H_{33}$

Pityrosporum ovale and P. orbiculare[67]. Obtusastyrene and dihydroobtusastyrene, 24, 25, are active against Candida



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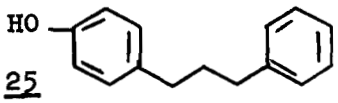

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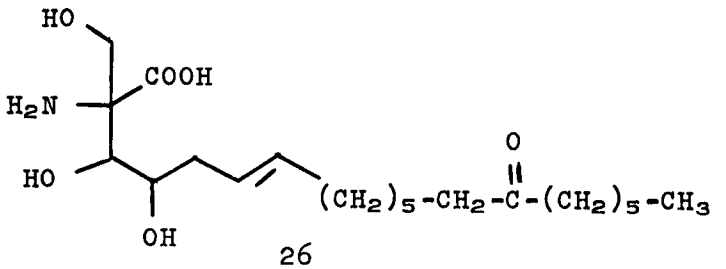
tropicalis and other yeasts at low concentrations[68]. Serinomycin a new antibiotic active against a variety of fungi is isolated from a Streptomyces and yields approximately 20% L-serine after hydrolysis with NH_4OH[69]. Gatavalin, a new peptide antibiotic isolated from Bacillus polymyxa var. colistinus, has some antifungal activity70. Myriocin, 26, has been isolated from the ascomycete Myriococcum albomyces;



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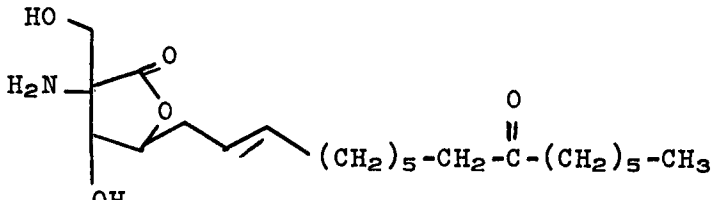
refluxing in tert-amyl alcohol resulted in anhydro myriocin 27;



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both compounds are highly active in vitro against Candida; however, anhydromyriocin showed activity against dermatophytes and was less toxic parenterally in mice and rats than myriocin[71]. A new polyene antibiotic, partricin (SPA-S-132), has been isolated from a strain of Streptomyces aureofaciens; esterification appeared to increase activity and reduce toxicity[72]. Methyl partricin is active against many strains of Candida and Aspergillus[73].

Physico-chemical constants, spectra and molecular models for clotrimazole, as well as methods of synthesis for other triphenyl methylazoles, have been described, including data for 112 triphenylmethylimidazoles. Structure activity relationships relating rate constants of acid hydrolysis and R_m values of 34 1-triphenylmethylimidazoles suggest that these two parameters are not sufficient for a complete description of antimycotic action. Ortho substituents were seen to produce a clear increase in activity[74].

Chemical and Physical Studies of Antifungal Agents - Streptomyces AY-B-265 incorporates L-phenylalanine and DL-tryptophane into antimycin A. Incorporation studies with 2-ring-C14-DL-tryptophane show that carbon 2 of the indole ring is incorporated into the 3 formamido carbonyl of antimycin in high yields[75]. The aromatic moiety of candicidin appears to be synthesized from glucose via shikimate to p-aminobenzoic acid which is then incorporated into candicidin. This synthesis was partially inhibited by L-phenylalanine, L-tryptophane and L-tyrosine[76]. A similar mechanism has been proposed with PABA as the direct terminal intermediate being incorporated into

the aromatic moiety of fungimycin[77].

The sugar moiety of YA-56 (related to phleomycin, bleomycin, zorbamycin) is 2-O-(3-O-carbamoyl-D-mannosyl) 6-deoxy-L-gulose[78]. Acid hydrolysates of YA-56X and Y show hydroxyalanine present in both antibiotics instead of threonine, a common constituent of phleomycins and bleomycin[79]. The nonpolyenic portion of the endomycin complex has been shown to be scopafungin[80]. The structure of the pentaene antibiotic chainin has been shown to be 28[81]. An improved synthesis of

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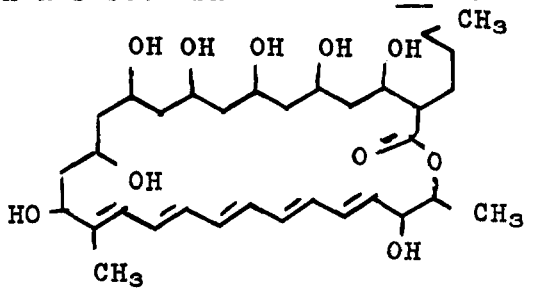

hexahydrospinamycin has been described[82]. The structure AN=NB and compounds with potential for conversion to that structure such as ANHNHB are characterized as diazene antibiotics and include the antibiotic hexahydrospinamycin. Structure activity studies show that higher antifungal activity is correlated with higher rate of reaction with glutathione, suggesting that the antibiotic action of diazenes may involve intracellular oxidation of glutathione to its disulfide[83]. The carboxylic group of amphotericin B has been esterified by reaction with diazomethane in tetrahydrofuran. The methyl ester thus obtained retained *in vitro* activity; N-acylation generally reduced activity[84]. Hydrochloride salts of methyl esters of a number of polyene macrolide antibiotics had greatly increased aqueous solubility[85]. *In vitro* and *in vivo* studies suggest similar activity for the methyl ester and the parent compound, but reduced toxicity for the methyl ester[86]. The filipin complex, as well as pimaricin, bind sterols that contain both a 3-β hydroxy group and a long alkyl side chain attached to the D ring, but interact weakly or not at all with cholesterol palmitate and 3-keto or 3-α hydroxy sterols[87,88]. Enhancing effect of *iso*-branched fatty acids on some fungicides may be due to increase in the permeability level of the plasma membrane based on evaluation of surface film studies[89]. Phosphorescence may be an extremely sensitive analytical procedure for griseofulvin since measurable phosphorescence can be obtained from solutions as dilute as 10^{-8} M[90].

Biological Studies of Antifungal Agents - Detailed chemotherapeutic evaluation of miconazole demonstrates broad-spectrum antifungal activity, both *in vitro* and *in vivo* in comparison with other antifungal agents[91]. Nine heptaene macrolide

antifungal antibiotics have been compared in experimental systemic C. albicans infections in mice. All showed similar activity subcutaneously but only amphotericin B and mycoheptin showed activity orally, which was related to their containing mycosamine as a nitrogenous moiety[92]. Administration of griseofulvin at levels of 1250 or 1500 mg/kg/day to pregnant rats during the period of organogenesis resulted in increased resorption sites, decreased litter size, and a syndrome of malformations[93]. Preclinical toxicological evaluation of haloprogin suggests absence of systemic toxicity in spite of percutaneous absorption. Some local irritation was seen but no indication of sensitization potential. Major metabolic products of haloprogin were 2,4,5-trichlorophenol and its sulfate conjugate[94]. Pyrrolnitrin is rapidly inactivated *in vivo* and metabolized *in vitro* to at least 4 oxidized pyrroles without antifungal activity. Of interest is the generation of a substituted maleimide by microsomal oxidation of the pyrrole[95]. Amphotericin B has been shown to potentiate the antifungal activity of rifampicin,[96,97] 5-fluorocytosine,[96] mycophenolic acid glucuronide,[98] tetracycline[98] and actinomycin D,[98] probably through the increased penetration of the agents through the cytoplasmic membrane. Similarily polymyxin B has been shown to potentiate the antifungal activity of tetracycline[99]. Oligomycin resistance in yeasts has been shown to be related to changed sensitivity of mytochondrial ATPase[100]. *Saccharomyces cerevisiae* resistance to nystatin has been related to altered sterol composition; perhaps in the cell membrane, based on lipid requirements and their effect on resistance[101]. Evaluation of spontaneous mutants of *C. albicans* resistant to 5-fluorocytosine, as well as resistant strains isolated from patients showed two classes of resistance: one unaffected by 5-fluorocytosine at the highest concentrations tested, and the other with low growth in the presence of high levels. Spontaneous mutation rates of susceptible strains to resistance were fairly high[102]. Calcium ions reversed the *in vitro* activity of heptamycin against several yeast strains[103]. Polyoxin D inhibits chitin synthetase which may be related to weakened walls noted in organisms growing in the presence of this antibiotic[104]. Cytochalasins A and D produce morphological changes of fungi, specifically branching and swelling of hyphal tips[105]. The effect of griseofulvin on mitosis in *Aspergillus* may be related to abnormal spindle formation[106]. 4-bromobenzyl isothiocyanate altered the redox state of intracellular NADP in *Candida*[107]. The antifungal antibiotic cerulenin inhibits sterol and fatty acid biosynthesis in cell-free systems of yeasts[108].

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Chapter 14. Antineoplastic Agents

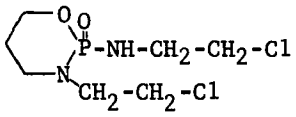
C.C. Cheng and Kwang Yuen Zee-Cheng
Midwest Research Institute, Kansas City, Missouri 64110

Introduction - There are now ten disseminated human cancers which are highly responsive to chemotherapy: Burkitt's lymphoma, choriocarcinoma, acute lymphocytic leukemia, Hodgkin's disease, lymphosarcoma, reticulum cell sarcoma, embryonal testicular cancer, Wilms' tumor, Ewing's sarcoma, and retinoblastoma. About 50% of these patients can be expected to achieve normal life expectancy by modern chemotherapy. The following twenty-five drugs have been repeatedly studied and recognized as having proven clinical value: mechlorethamine hydrochloride (nitrogen mustard), cyclophosphamide (cytoxan, endoxan), melphalan, chlorambucil, busulfan, thioTEPA, dibromomannitol, methotrexate, 5-fluorouracil, 6-mercaptopurine, thioguanine, cytosine arabinoside (cytarabine), 6-azauridine, vincristine, vinblastine, actinomycin-D, daunorubicin, adriamycin, mithramycin, streptozotocin, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, carmustine), hydroxyurea, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide, procarbazine hydrochloride, and o,p'-DDD (mitotane).[1]

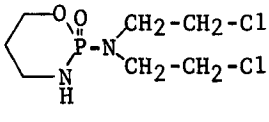
Reviews on preclinical and clinical evaluation of antitumor agents,[2,3] immunosuppressive compounds,[4] mechanism of action in cancer chemotherapy,[5] antineoplastic antibiotics,[6] and other newer agents[7] were recently published.

Current attention in the development of antineoplastic compounds is being focused on agents that would be active against slow-growing tumors, since most of the present day drugs are mainly effective against rapid-growing cancers.

Alkylating Agents - Isophosphamide (I), an analog of cyclophosphamide (II), inhibits Lewis lung carcinoma, Ehrlich ascites sarcoma, and Yoshida sarcoma.



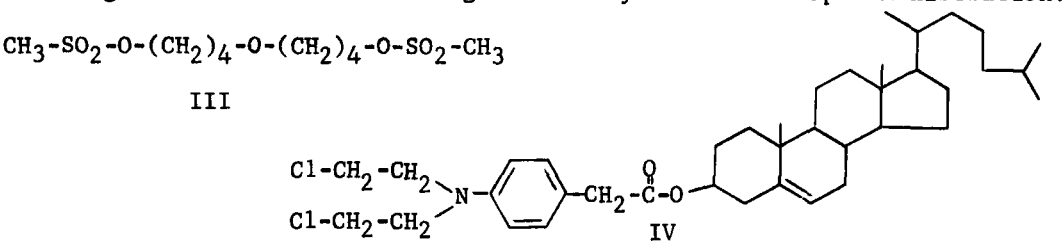
I



II

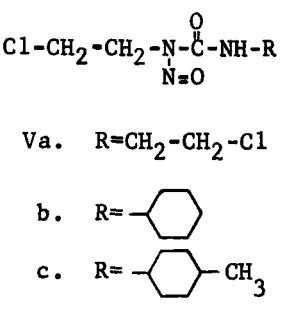
I generates the cytotoxic aldehyde acrolein in vitro,[8] the latter has been proposed as a component of a universal cell-growth regulatory system.[9] Clinically it is active in oat cell tumors of the lung, ovarian cancer, breast cancer, and lymphomas.[10] Toxicities of both compounds I and II are similar: They cause cardiovascular alterations in rhesus monkeys with I being twice as potent.[11] The hypotension and brodycardia observed after administration of these compounds may be due to a direct nonspecific cardiac depression in conjunction with possible histamine release.[11]

Bis(4-methanesulfonoxybutyl) ether (III) and many related δ-oxygen-substituted butyl ethers are effective inhibitors of the Walker rat carcinoma.[12] Phenesterin, (IV), an alkylating cholesterol ester, is at least as effective as cyclophosphamide and about three times that of chlorambucil. The drug is more effective when given orally than sc or ip administration.[13]



III IV

Nitrosoureas - Methyl-CCNU (Vc) is believed to be a better drug than CCNU (Vb), which is in turn more active than BCNU (Va). The use of substituent constants and regression analysis suggest that a study of more hydrophilic nitrosoureas should uncover more potent and less toxic drugs.[14] CCNU chemically modifies proteins mainly via cyclohexyl-carbamoylation of lysine residues and modifies nucleic acids via alkylation. The dual capacity of Vb may explain its broad cytotoxicity and its activity against tumors which are resistant to conventional alkylating agents.[15] Clinically these drugs are effective against recurrent brain tumors[16] but ineffective in the treatment of either end-stage breast cancer[17] or bronchogenic carcinoma.[18]

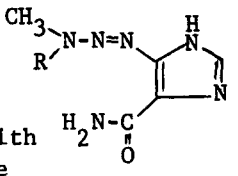


Va. R=CH_2-CH_2-Cl

b. R=

c. R=

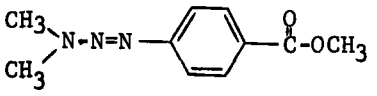
5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (VIa, DIC) and related compounds - Mode of decomposition of DIC was studied in Bacillus subtilis[19] and in mamalian cell cultures.[20] In the presence of light, DIC decomposed into dimethylamine and 5-diazoimidazole-4-carboxamide, the latter enters the cells and interacts with nucleic acids in an obscure manner. When light is excluded, DIC forms 5-aminoimidazole-4-carboxamide and a methyl carbonium ion, the latter interacts with cell DNA. DIC is lethal to both proliferating and nonproliferating cells. It is metabolized by tumor tissue in a manner similar to that in normal tissue and the VIb intermediate decomposes spontaneously to generate a methylating agent.[21] Activity has been demonstrated for patients with Hodgkin's disease.[22] Although DIC is markedly effective against a variety of murine neoplasms, it is without effect against advanced acute lymphocytic leukemia in children.[23]



VIa. R=CH_3

b. R=H

Derivatives of 1-phenyl-3,3-dimethyltriazene have also shown anti-leukemic activity in mouse leukemia L1210. A benzoate VII, in particular, possesses a better therapeutic index than DIC.[24]

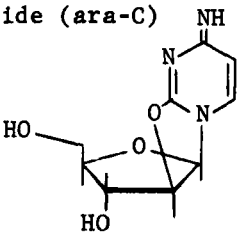


VII

Pyrimidines and Azapyrimidines - Direct synthesis of 5-fluoropyrimidines and their nucleosides, by treatment of the corresponding pyrimidines or nucleosides with trifluoromethyl hypofluorite, was achieved.[25-27] 5-FU is preferentially concentrated (ca. 11 times) in the brain of C57BL/6 mice with intracerebral glioma than in normal brain of mice.[28] 5-Fluoro-2'-deoxyuridine-5'-monophosphate, the active metabolite of 5-FU, persists in all tissues of mice with L1210 leukemia for 72 hrs, with highest concentration in the small intestine.[29] 5-Fluoro-2,2'-anhydro-arabinocytosine is markedly active against leukemia L1210.[30] 5-Fluoro-3'-deoxyuridine and 5-trifluoromethyl-3'-deoxyuridine, unlike their 2'-deoxyribonucleoside counterparts, are inactive against HeLa, L5178Y, and Novikoff hepatoma cells.[31] 5-Diazouracil, which irreversibly inhibits dihydrouracil dehydrogenase in pyrimidine biosynthesis, promotes the synthetic utilization and retards the catabolism of thymine.[32]

^{14}C study in patients revealed that 5-azacytidine uptake by tumor tissue is greater than that by surrounding normal tissue and the drug is incorporated into tumor RNA but not into DNA.[33] 5-Azacytidine depresses polyamine synthesis in L1210 leukemic mice.[34] This drug induces objective remissions in patients with breast cancer, melanoma, and colon cancer.[35]

Cyclocytidine (VIII, 2,2'-anhydro-β-D-arabinofuranosylcytidine) is active against a variety of tumors (adenocarcinoma 755, Nakahara-Fukuoka sarcoma, ascites sarcoma 180, Ehrlich ascites carcinoma, L1210 leukemia, C1498 leukemia) in mice.[36] In general, VIII possesses greater therapeutic index and lower cumulative toxicity than cytosine arabinoside (ara-C) but has little activity against reticulum cell sarcoma.[37]



VIII

Hexamethylmelamine is useful for the treatment of adenocarcinoma of the lung, carcinoma of the ovary, and Hodgkin's disease.[38] It is inactive against acute leukemia resistant to standard chemotherapeutic agents.[39] The mechanism of action of this compound is not yet known.

Purine Nucleosides - The mode of inhibitory action of 6-methylthiopurine riboside (6-MeMPR) may be due to inhibition of purine biosynthesis de novo by 6-MeMPR phosphate.[40] The β-anomer of 2-chloro-2'-deoxyadenosine and related compounds are more potent inhibitors than the corresponding α-anomers against tumor cell growth.[41]

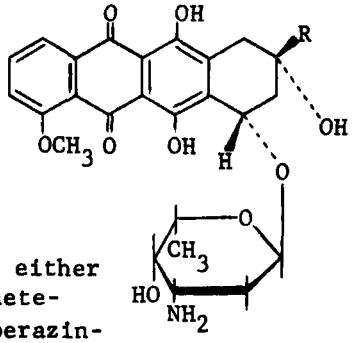
Folic Acid Antagonists - A practical synthesis of homofolic acid from

6-hydroxy-2,4,5-triaminopyrimidine and 1-acetoxy-4-[N-acetyl-(p-carbethoxyphenyl)amine]-2-butanone was reported.[42] A study on the mechanism of action of the antileukemic drug tetrahydrohomofolic acid (H_4HF) revealed that H_4HF may interfere with the conversion of orotic acid to deoxyuridine in pyrimidine biosynthesis and that thymidylate synthetase, shown to be the most sensitive enzyme to H_4HF in vitro, may not be the primary target of the drug in vivo.[43]

N^5-Formyltetrahydrofolic acid reverses the action of methotrexate (MTX) in L1210 leukemia cells.[44] Dichloromethotrexate displays some antitumor activity in hepatocellular carcinoma. This dichloro derivative is concentrated in the liver to a greater extent than MTX.[45]

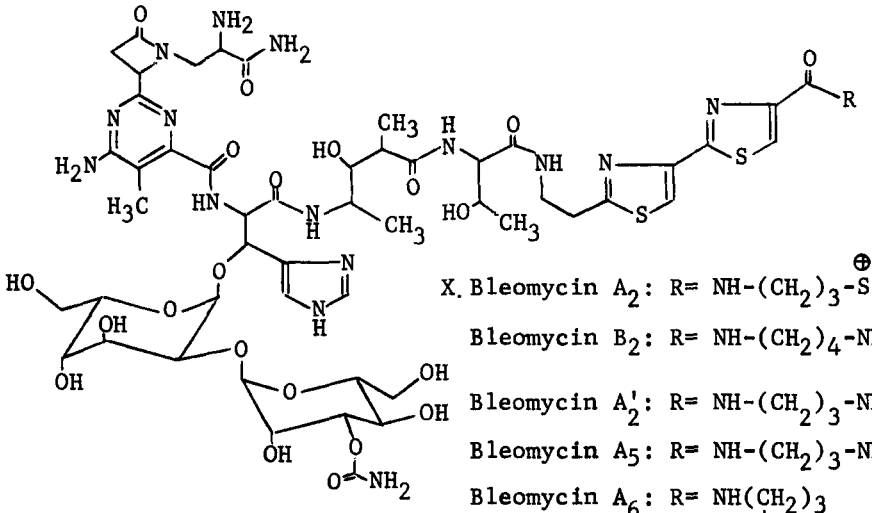
Actinomycin D (Act-D) - The association of act-D and different deoxyribodinucleotides were studied as model complexes for the interaction of act-D and DNA. All of the deoxyribonucleotides containing guanine will complex with act-D, with a preference for G-C sequences of DNA as potential binding sites.[46] Act-D lactam was synthesized.[47]

Adriamycin and Daunorubicin - Daunorubicin (IXa) and DNA interact predominately through intercalation.[48] The binding of IXa to DNA involves more than one class of sites. The amino sugar residue of IXa is involved in the stabilization of DNA complex.[49] Daunorubicinol (IXb), the metabolite of IXa, is as effective as its parent compound against leukemia P388 but not as effective against leukemia L1210 in mice.[50] On the other hand, adriamycin (IXc) does not undergo such conversion,[51] which may account for the fact that IXc is consistently more effective than IXa in transplanted mammary carcinoma[52] and other tumor systems. The iv route rather than ip or sc administration of these drugs are recommended for achieving maximum effectiveness in solid tumors.[53] The cytotoxic effect of both IXa and IXc persisted in the host cells for 20-30 hours after administration.[54]



IXa. R=CO-CH_3
b. R=CHOH-CH_3
c. R=CO-CH_2OH

Adriamycin is a useful chemotherapeutic adjunct in the treatment of Ewing's sarcoma[55] but is quite toxic to patients with acute myeolocytic leukemia.[56] The adriamycin-resistant tumor is also cross resistant to daunorubicin and vincristine.[57,58] The cardiotoxicity of these antibiotics may be reduced by the pretreatment of either of the following chelating agents: ethylenediaminetetraacetic acid (EDTA), or (±)-1,2-di(3,5-dioxopiperazin-1-yl)propane (ICRF 159).[59] Some amino and methyl ketone derivatives of daunorubicin still retain the original antitumor activity.[60]



X. Bleomycin A_2: R= $NH-(CH_2)_3-\overset{\oplus}{S}(CH_3)_2\overset{\ominus}{X}$

Bleomycin B_2: R= $NH-(CH_2)_4-NHC(=NH)-NH_2$

Bleomycin A_2': R= $NH-(CH_2)_3-NH_2$

Bleomycin A_5: R= $NH-(CH_2)_3-NH-(CH_2)_4-NH_2$

Bleomycin A_6: R= $NH(CH_2)_3$ with $(CH_2)_3-NH_2$ and $NH-(CH_2)_4-NH$

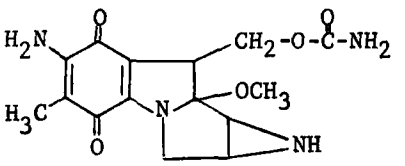
Bleomycins - Complete structures of bleomycins have been elucidated and established as X.[61] Synthesis of β-amino-β-(4-amino-6-carboxy-5-methyl-pyrimidin-2-yl)propionic acid, a heterocyclic component of bleomycin, was reported.[62]

Higher concentrations of blemomycin were accumulated in carcinoma than in sarcoma. In addition, higher concentrations of its active form were noted in the lungs and skin of old mice than in those of young ones.[63] Bleomycin may have affected cellular sulfhydryl or disulfide groups, or affected the availability of essential metals in the organs that concentrated this antibiotic.[64]

Bleomycin A_2 exerts a lethal effect on mamalian cells and also induces resistance in these cells.[65] At high dosages, bleomycins can significantly prolong the rejection time of skin graft in animals, yet this altered graft rejection behavior was not due to specific immunosuppression,[66] as generally believed. Histological examination of the mouse small intestine reveals a profound antimitotic action of bleomycin. This action takes place at the late S to early G_2 transition.[67,68] Results from clinical study with malignant lymphomas and testicular tumors seem promising;[69] objective regression in patients with metastatic large bowel cancer, however, was not noted.[70]

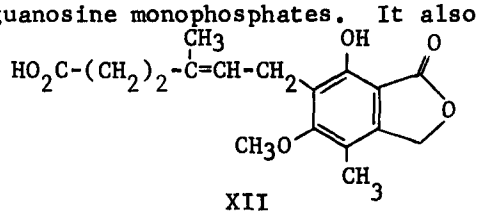
Mitomycin C - A claim that the carbamyl group and the aziridine ring of mitomycin C (XI) and related compounds may be replaced by other acyl groups without loss of biological activity[71] has been supported by the fact that a series of benzoquinone derivatives containing side chains capable of alkylation after bioreduction displayed growth-inhibitory activity against adenocarcinoma ascites cells and are potent inhibitors of DNA and RNA synthesis

in neoplastic cells.[72]



XI

Mycophenolic Acid - Originally isolated from a culture of Penicillium glaucum (a mold found on corn) and subsequently from Penicillium stoloniferum, mycophenolic acid (XII), inhibits the growth of Mecca lymphosarcoma and the solid and ascites forms of Walker carcinosarcoma 256 and moderately inhibit Gardner lymphosarcoma, C3H mammary carcinoma, and adenocarcinoma 755. However, it is inactive against leukemias L1210 and C1498.[73] It is synergistic with cyclophosphamide against experimental lymphosarcomas.[73] Compound XII interferes in the interconversion of inosine, xanthosine, and guanosine monophosphates. It also inhibits IMP dehydrogenase and GMP synthetase.[74] Compound XII is readily absorbed from the digestive tract and rapidly conjugated to mycophenolic acid β-glucouronide.[75] Preliminary clinical trials indicated that, although toxicity is rather low, this acid does not cause tumor regression in patients with a wide range of advanced malignant tumors.[76,77] Some derivatives of mycophenolic acid, including its glucouronide, were reported to possess antitumor and immunosuppressive activity.[78,79]



XII

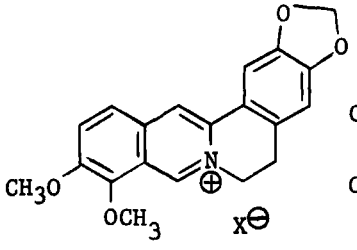
Neocarzinostatin - The primary structure of the antitumor protein neocarzinostatin, isolated from Streptomyes carzinostaticus var. F-41, was characterized as an acidic single-chain peptide. The protein contains 109 amino acid residues (mol wt ~ 10,700). The amino acid composition is unusual in its high content of alanine, glycine, serine, and threonine but no histidine or methionine.[80] Neocarzinostatin is effective against ascitic sarcoma 180, ascitic leukemia SN36, and leukemia L1210. It also rapidly and differentially affected Burkitt's lymphoma cells in culture.[81] Deamination or acylation of this protein at the terminal amino group (alanine) decreases toxicity with retention of antitumor activity in mice.

Neocarzinostatin is unstable above 37°. It is inactivated by tissue homogenate, serum or SH-containing compounds accompanied with copper.[82] It inhibits both DNA and RNA synthesis but protein synthesis remains unchanged.[81]

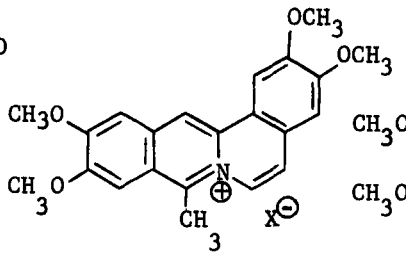
Berbinium Salts, Coralyne, and Nitidine - Among the berbinium salts, the sulfate and chloride of berberine (XIIIa,b) are cytostatic toward Ehrlich or lymphoma ascitic tumor cells in vitro.[83] Coralyne salts (XIVa,b) show significant activity against leukemias L1210 and P388 in mice.[84] The corresponding dihydro compound XV, however, is devoid of antileukemic

activity, indicating that the planarity of the molecule may be of importance. UV spectral studies reveal that a stable complex is formed upon the interaction of DNA in vitro.

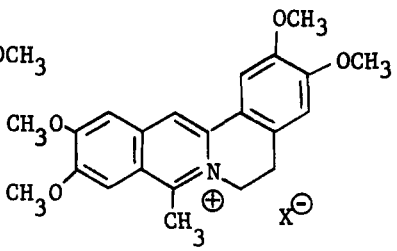
Salts of nitidine (XVIa,b) and 5,6-dihydro-6-methoxynitidine (XVII), a new alkaloid from Fagara macrophylla, possess high cytotoxicity and activity against leukemias L1210 and P388.[85,86] Another new alkaloid, fagaronine (XVIc), isolated from Fagara zanthoxyloids Lam. (Rutaceae), also shown good activity against leukemia P388.[87] Structures of these compounds conform to an earlier proposed N-O-O triangular pharmacophore hypothesis for antileukemic activity.



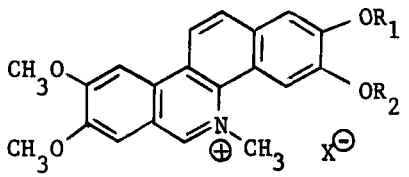
XIIIa. X= ½ SO_4
b. X= Cl



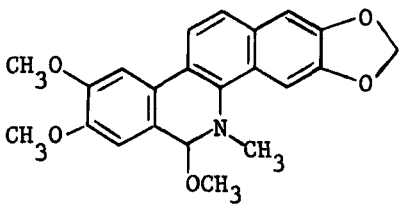
XIVa. X= $C_2H_3SO_5$
b. X= Cl



XV

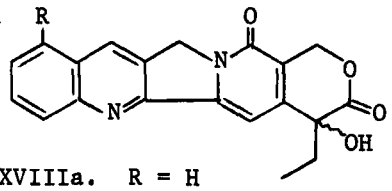


XVIa. $R_1 + R_2$= CH_2, X = CH_3SO_4
b. $R_1 + R_2$= CH_2, X = Cl
c. R_1 = H(or CH_3), R_2= CH_3(or H), X = Cl



XVII

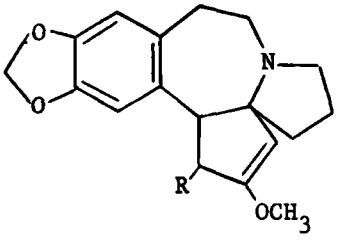
Camptothecin - Additional synthetic methods for the preparation of d,l-campthothecin (XVIIIa) and related derivatives were reported.[88-91] A new alkaloid, 9-methoxycamptothecin (XVIIIb), was isolated along with camptothecin as the major product from Mappia foetida Miers.[92] Camptothecin blocks the ribosome formation from 32S RNA to 28S RNA, inhibits synthesis of both nucleoplasmic and polyribosomal m-RNA in HeLa cells,[93] induces nucleolar structural changes in ME-180 tissue culture cells[94] and causes intracellular degradation of HeLa cells and adenovirus type-2 DNA.[95] The progression of late S or early G_2 cells into mitosis of



XVIIIa. R = H
b. R = CH_3O

mammalian cells (leukemia L1210 cells and asynchronous and synchronous DON cells) is most sensitive to this alkaloid.[96] Clinical study of camptothecin in the treatment of advanced gastrointestinal cancer, advanced disseminated melanoma or a variety of carcinomas failed to show much activity.[97-99]

Cephalotaxus Alkaloids - The alkaloid cephalotaxine (XIXa) and a number of its esters were isolated from the seed of Cephalotaxus harringtonia. Although XIXa is inactive, harringtonine (XIXb), isoharringtonine (XIXc), homoharringtonine (XIXd), and deoxyharringtonine[100] (XIXe) possess anti-tumor activity against leukemias L1210 and P388.[101] Synthesis of XIXa and



XIXa. R = OH

b. R = O_2C-C(OH)(CH_2-CO_2CH_3)-$(CH_2)_2$-$C(CH_3)_2OH$

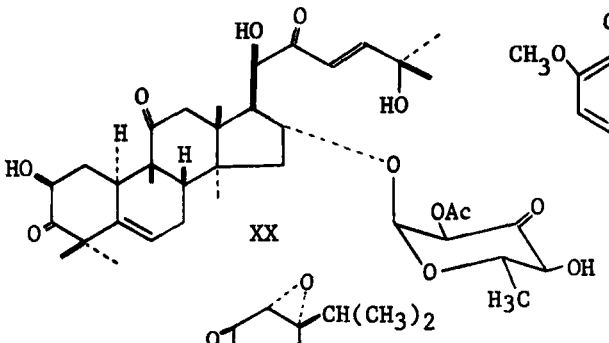
c. R = O_2C-C(OH)[CH(OH)-CO_2CH_3]-$(CH_2)_2$-$CH(CH_3)_2$

d. R = O_2C-C(OH)(CH_2-CO_2CH_3)-$(CH_2)_3$-$C(CH_3)_2OH$

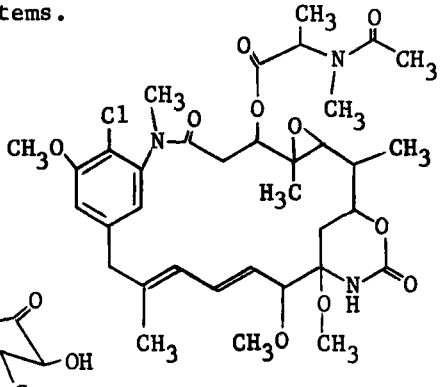
e. R = O_2C-C(OH)(CH_2-CO_2CH_3)-$(CH_2)_2$-$CH(CH_3)_2$

related compounds have been accomplished.[102]

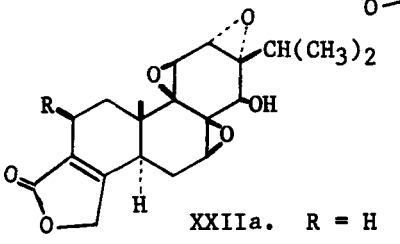
Other Plant Products - Datiscoside[103] (XX), a cucurbitacin glycoside from Datisca glomerata, maytanisine[104] (XXI), an ansa macrolide from Maytenus ovatus, as well as triptolide[105] (XXIIa) and tripdiolide[105] (XXIIb), diterpenoid triepoxides from Tripterygium wilfordii, possess activity against a number of standard animal tumor systems.



XX



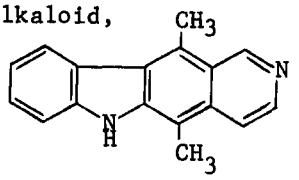
XXI



XXIIa. R = H

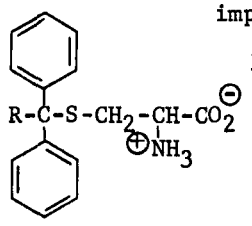
b. R = OH

Ellipticine - Ellipticine (XXIII), a cytotoxic plant alkaloid, kills cells in all phases of the cell cycle. Cells in M and G_1 phases are much more sensitive to XXIII than in S and G_2 phases. XXIII inhibits DNA and RNA syntheses more than protein synthesis.[106] Two new syntheses of XXIII were reported.[107,108]



XXIII

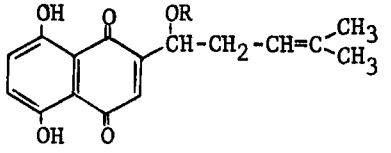
S-Trityl-L-cysteine (3-Tritylthio-L-alanine) - This amino acid (XXIV) is active against leukemia L1210. The internal zwitterion form of XXIVa is important for biological activity since substitution or modification at either the amino or the carboxylic acid group decreases the activity.[109] S-Trityl-D-cysteine and O-trityl-L-serine possess low but definite antileukemic activity. Also, S-(2-naphthyldiphenylmethyl)-L-cysteine (XXIVb) possessess better activity than XXIVa but the corresponding 1-naphthyl isomer XXIVc is inactive.



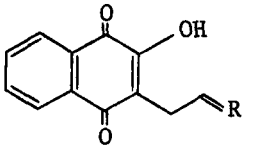
XXIVa. R = C_6H_5
b. R = 2-naphthyl
c. R = 1-naphthyl

The low solubility of XXIVa in water limits its absorption. The main site of compound concentration in rats, dogs, and monkeys is the liver. Appreciable concentrations was also found in the kidney of the monkey.[110]

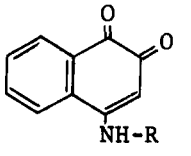
Naphthoquinones - Arnebin (XXVa), a naphthazarin derivative isolated from the roots of Arnebia nobilis, inhibits rat Walker carcinosarcoma 256.[111] This compound bears a close structural resemblance with shikonin (XXVb) and both compounds may be biogenetically related. Compound XXVb, isolated from Lithospermum erythrorhizon Sieb et Zucc., is active against human carcinoma of the nasopharynx (KB). Several lapachol derivatives[112] (XXVIa and XXVIb) and some 4-amino-1,2-naphthoquinones[113] (XXVII) have also been claimed to possess antitumor activity.



XXVa. R = $CO-CH=C(CH_3)_2$
b. R = H

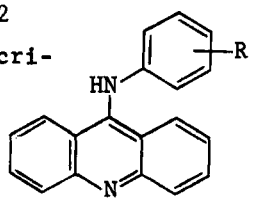


XXVIa. R = CBr_2
b. R = $C(CH_3)_2$



XXVII

9-Anilinoacridines - Some 1-nitro-9-(substituted amino)acridines are cytostatic.[114] A series of 9-(substituted anilino)-acridines (XXVIII) wherein R represents an electron-donating group substituted at positions 3' and 4' are active against leukemia L1210.[115]



XXVIII

Thiosemicarbazones - A number of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones were prepared and their antineoplastic activity measured. In general, the pyridine derivatives are better inhibitors than the isoquinoline derivatives against lymphomas.[116,117]

Chlorphenesin - (3-p-Chlorophenoxyl-1,2-propanediol)--This compound has shown antineoplastic activity in a number of experimental tumor systems, including virus-induced murine leukemias and several transplantable tumors. It is essentially nontoxic at therapeutically active levels. Chlorphenesin may act by enhancing cell-mediated immune responses of the host. Clinically, chlorphenesin is valuable in the treatment of squamous cell carcinoma of the skin.[118] Other related oxygen containing compounds, such as hydroxylated straight chain aldehydes (e.g. L-erythro-α,β-dihydroxybutyraldehyde)[119] and glyceryl ethers of fatty alcohols,[120] have also shown antineoplastic activity.

Inhibitors of t-RNA Methyltransferase - A detailed account of the relationship of elevated level of t-RNA methyltransferase activity (which results in the formation of abnormally large amounts of C-, N-, and O-methylated nucleosides in t-RNAs) and tumor induction was presented.[121] Nicotinamide[122] and some related analogs as well as certain cytotoxic purine ribosides[123] are found to inhibit t-RNA methyltransferase activity. The claim that appropriately designed nicotinamide analogs may possess antitumor activity[122] was substantiated by the fact that 6-dimethylaminonicotinamide is active against the solid Friend virus (FV) leukemia.[124] Certain compounds having adjacent oxygen functions, or compounds having similar interatomic distances between two oxygen atoms, may interfere with the undesired activity of t-RNA O-methyltransferase and inhibit the process of abnormal cell proliferation.[125]

New Conceptions and Hypotheses

Alternation in the cell membrane that results in increased internal concentrations of nutrients, which interrupt the regulation of cell growth, has been postulated as a factor of malignant growth.[126] Certain protease inhibitors are believed to interfere with fibrinolysis in tissues. Since degradation of body fibrin by fibrinolysins creates conditions that promote growth and spread of tumors, properly designed protease inhibitors might be useful in the treatment of solid tumors.[127]

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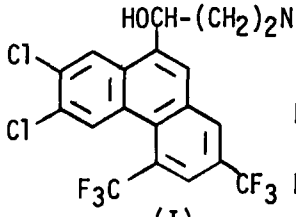
Chapter 15. Antiparasitic Agents

M. Hoffer and C. W. Perry, Hoffmann-La Roche Inc., Nutley, N. J. 07110

Introduction - A table of drugs for parasitic infections has appeared.[1] The proceedings of the Inter-American Malaria Research Symposium, San Salvador, El Salvador, Nov. 1-4, 1971 were published,[2] including articles by L. J. Bruce-Chwatt, D. F. Clyde and R. D. Powell on current drug therapy and the development of new drugs. Abstracts for the Annual Meeting of the Society of Protozoologists, Minneapolis, Minn., August 28-September 1, 1972, can be found as a supplement to the Journal of Protozoology.[3] The WHO Chronicle contains reports on malaria eradication.[4] The Amer. Vet. Med. Assoc. held a symposium[5] on problems of new animal drug development.

Antimalarials - A model in vitro system for testing susceptibility of human parasites to antimalarial drugs was proposed.[6] Structure-activity correlations for 2-phenylquinoline-4-carbinol antimalarials were obtained by the Free-Wilson method.[7] An extensive study of tissue distribution and urine excretion of chloroquine was reported.[8] Observations on the mode of action of chloroquine and quinine in blood stages of P. berghei were reported by Warhurst, Homewood and Bagaley.[3] Development of resistance and cross-resistance to antifolic antimalarials was studied in a variety of bacteria.[9] The antimalarial primaquin caused a total inhibition of protein synthesis in B. megaterium.[10]

Parallelism in reversible coenzyme Q_{10}-inhibition and antimalarial properties was demonstrated in substituted quinolinequinones[11] and naptho-quinones.[12] Extensive structure-activity relationships in phenanthrene-9-amino alcohols (very active I, curative at the 5 mg/kg level and still active at the 1.25 mg/kg level in the P. berghei-mice screen)[13-15] and in



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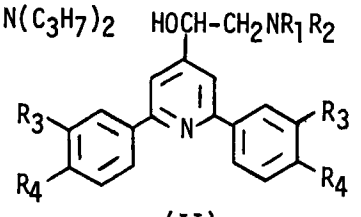
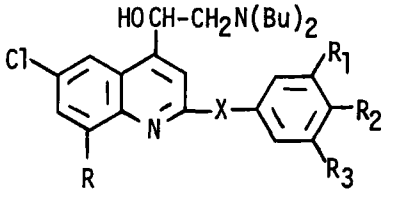


(II)

2,6-diaryl pyridyl-4-methanols (best II, R_2=R_3=H, R_1=4-heptyl, R_4=CF_3, and R_2=R_3=H, R_1=1-butyl, 2-butyl or 4-heptyl, R_4=Cl, curative at the 40 mg/kg level and active at the 20 mg/kg level) were published.[16,17] Introduction of the bridge X=CO,CH_2,CF_2 to reduce phototoxicity in quinoline carbinol antimalarials (III) also reduced



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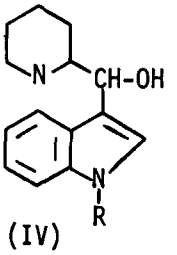

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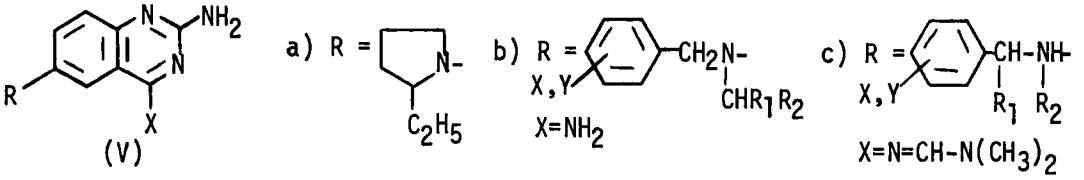
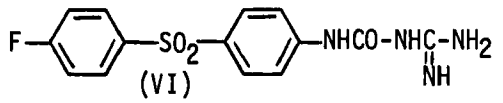


activity.[18] Among 1-aryl-7-azaindole-3α-piperidinemethanols, IV with R=p-chlorobenzyl showed half the activity of quinine.[19] Novel antifolates (V) with R=$C_6H_5CH_2NH$-, X=OH have shown activities in drug resistant malaria and Tryp. cruzi infections.[20] Compound Va has shown 210 times the activity of quinine,[21] while compounds Vb were potent antimalarials in evaluations with P. berghei, galinac, cinnomogli, and knowlesi (most interesting: X,Y=3,4 Cl_2, R_1=R_2=H) and they also show promise against Tryp. cruzi.[22] Compound Vc is claimed in a recent patent.[23] 2,4-Diamino-5-(3,4,5-trimethoxyphenoxy)-pyrimidine (related to trimethoprim) showed 100% supression of oocysts at a conc. of 0.1% in the mosquito-screen, but analogs were inactive.[24]

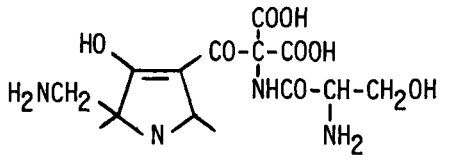
Among a series of guanidine derivatives of diphenylsulfones, VI was found best.[25] Diformyldiaminodiphenylsulfone (DFD) was proposed as prophylactic against falciparium malaria.[26] Its pharmacology was studied in dogs and monkeys[27] and in man.[28]



Chlorinated lincomycin analogs were evaluated against chloroquine resistant falciparium malaria.[29] The antibiotic clindamycin phosphate (Cleocin phosphate, U28508), a lincomycin analog, shows antimalarial activity.[30] Synthesis and resolution of 1'-demethyl-4'-depropyl-4' (R)- and -(S)-n-pentylclindamycin hydrochloride (U24729A) has been achieved.[31] Some novel lincomycin derivatives are obtained by adding alkylprolines to a fermentation broth of Streptomyces lincolnensis.[32]

Antitrypanosomal agents - Thymidine kinase from Tryp. brucei rhodensiense was isolated.[33] Roitman and Roitman observed increased growth inhibition effects of trypanocidals (ethidium bromide, acriflavine, quinacrine, melarsen, and tryparsamide) at 37° as compared with 28° in Leptosoma pessogi.[3] Cosgrove and Hajduk indicate that hydroxyurea prevents multiplication of T. equiperdium by irreversibly inhibiting DNA but not RNA and protein synthesis.[3] Morales, Schaefer, Keller and Meyer isolated DNA from Leishmania tropica and induced dyskinetoplasty by acridine and ethidium bromide but not by proflavin and 5-amino-acridine.[3] A pathway for terminal electron transport of the respiration metabolism in Tryp. mega was proposed by Ray and Cross.[3]

Vermicillin, a novel antibiotic from Penicillium vermiculatum was found active against Tryp. cruzi and Leishm. brasiliensis.[34] Antibiotic K16 (VII) is active against protozoa, esp. Tryp.[35]



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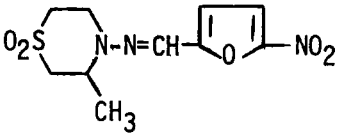

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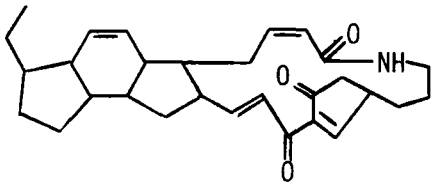


(IX)

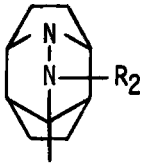
Isolation and characterization of the antiprotozoal, pigmented antibiotic, trypanomycin, probably a 4,5,8-trihydroxyanthraquinone, were described.[36] 3-Methyl-4-(5'-nitrofurfurylideneamino) tetrahydro-4H-1,4-thiazine-1,1-dioxide (nifurtimox, lampit, BAY 2502), (IX), is highly promising in treatment of acute and chronic Chagas-disease.[37-47]

Antitrichomonals - Trimonil (Trimagill), a micronized aluminum salt, was effective in topical application against Trich. vag., Hemophilus vag. and Candida (monilia) albicans and was recommended with or without additional chemotherapeutic treatment.[48] A new synthesis of niridazole allowed the preparation of a series of analogs which were evaluated in trichomoniasis of mice.[49] The novel antibiotic ikarugamycin (IX) was reported active against Trichomonas.[50] Some 2-styryl-5-nitroimidazoles were comparable to metronidazole against Trichomonas but in general inferior to the latter in other protozoal infections.[51] Flunidazol (MK915) was effective locally at 0.125-1 µg/ml and orally at 20-50 mg/kg levels, also as an amebicide in rats and hamsters.[52] Nitroimidazoles were discussed from constitutional and physicochemical standpoints with respect to antitrichomonal activity.[53] 1,1'-Dimethyl-2,2'-biimidazoles gave upon nitration a variety of compounds which were evaluated against Trich. vag. and Entamoeba hist. Compounds with two nitro groups were found generally more active than compounds with only one. For activity against Trich. at least one of the nitro groups must be in the 5 position.[54] The newer patent literature claims 2-(5-nitro-2-furyl)vinyl-thieno(3,2-d)pyrimidines,[55] 5-nitro-imidazolylvinyl-amino-1,3,4-oxadiazoles,[56] and 2-methyl-1-substituted-5-hydroxyalkyl-4-nitroimidazoles[57] as active antitrichomonals.

Coccidiostats - Comparative studies on established coccidiostats can be found in various publications.[58-63] Efficacy[64] and safety[65] (>0.005, <0.01% in feed) of 3-nitro-4-hydroxyphenylarsonic acid (Roxarsone) were reexamined. Deposition and clearance of Rofenaid in eggs were investigated.[66]

p-Dimethylaminobenzonitrile shows activity at 0.0125% in feed if potentiated with antifolates (best ormethoprim at 0.0075% in feed).[67] Certain mercapto and sulfinyl derivatives of thiamine are coccidiostats.[68-72] The newer patent literature claims 9,10-diazatetracycloundecene compounds (X),[73] nitrofurfuraldehyde sulfonylhydrazones,[74] 2-phenyl-as-triazine

diones,[75] 2-phenyl-azacytosine derivatives,[76] nicarbazine in combination with 1,3-bis-(p-chlorobenzylideneamino)guanidine,[77] tris(p-chlorobenzylideneamino)guanidine,[78] and 4-nitrothiophene-2-sulfonamides and 5-nitrothiophene-2(or 3) carboxamides[79] as active coccidiostats.

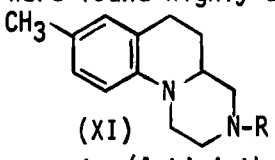


(X)

Amebicides - Metronidazole seems to be the choice as to current therapy of amebiasis,[80-83] dracunculiasis,[84] and giardiasis.[85] It proved superior to nitrimidazine (1-(N-β-ethylmorpholine)-5-nitroimidazole, naxogin) and tinidazole (fosigyn) and about equal to Ro 7-0207 (α-chloromethyl-2-methyl-5-nitroimidazole ethanol) in amebic liver abscess.[86] The pharmacology of panidazole was studied in animals.[87]

The newer patent literature claims 3-dimethylamino-9-(aminoalkyl) thioacridines[88] and 4-hydroxy-1-substituted-1H-thieno(2,3-c)pyrazoles[89] as amebicides.

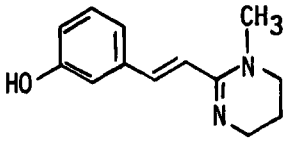
Schistosomacides - Some comparative studies on established schistosomacides (niridazole, lucanthone, astiban) can be found in the newer literature,[90] including effects on the oxidative metabolism.[91] 2-Diethylaminoethylamino-4,6-diamino-5-nitrosopyrimidine was found active.[92,93] Some 2,3,4,4a,5,6-hexahydro-1H-pyrazino{1,2-a}quinolines (XI), screened in mice at 50 mg/kg, were found highly effective in monkeys in single doses of 50-75 mg/kg.[94]



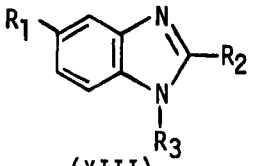
(XI)

2,4-Di(4-arylpiperazino)-3-pentanones[95] and 2-acylimino-5-nitro-N-acyl-4-thiazoline-3-acetamides[96] are claimed in the newer patent literature.

Other anthelminthics - New experiences with pyrantel pamoate (Antiminth, Cobantrin) abound.[97-101] Pyrantel and morantel analogs were tested in sheep to correlate primary mouse screenings. Activities were also found in dihydrothiazine analogs of the two anthelminthics.[102] Of particular interest is the morantel analog CP 14445 (XII) which was found highly effective against Trichura muris.[103-104] Combendazole (XIII, R_1=CH(CH$_3$)$_2$CONH, R_2=NHCOOCH$_3$, R_3=H) was effective against lungworms.[105,106] Thiabendazole (XIII, R_1=R_3=H, R_2= ) was inferior to



(XII)



(XIII)

(XIV)

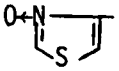
diethylcarbamazine[107] in experimental Toxicana caris infection in mice[108] but compared favorably (66 mg) with levamisol (8 mg) under commercial feedlot conditions against mixed worm infections in cattle.[109] Lobendazole, SKF 24529 (XIII, R_1=R_3=H, R_2=NHCOOC$_2$H$_5$) is recorded as new.[110] Methyl-1-{methoxycarbonylthiocarbamoyl}benzimidazole carbamates (XIII, R_1=H, CH$_3$, OCH$_3$, NO$_2$; R_2=NHCOOR, R_3=CSNH-A) are claimed to have anthelminthic in

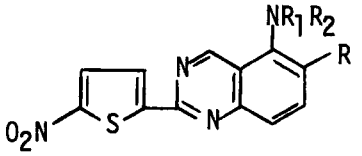
addition to fungicidal and mite ovicidal properties.[111] 2-Sulfonylalkylbenzimidazoles (XIII, R_2=CH_2SO_2R) showed activity against Nematospiroides dubious only if R_1=NO_2.[112] Alkylimidazopyridyl-carbamates (XIV) are claimed to have broad spectrum anthelminthic and antifungal activities.[113]

New salicylanilides related to the well established rafoxanide[114] are dioxamide (2-acetoxy-4'-chloro-3,5-diiodobenzanilide)[115] and terenol (4'-bromo-2,6-dihydroxybenzanilide).[116] Benzothiadiazole salicylamides are claimed to be cesticidal agents and anthelminthics.[117]

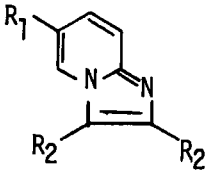
$Cl_2C{=}CHOPOOCH_3$ (XV), OR

Homologues of the acetylcholinesterase inhibitor dichlorphos (XV, R=CH_3)[118] displayed maximum activity against pinworms[119,120] when R=n-heptyl (vincophos, Shell 15803)[121,122] and against tapeworm with R=n-decyl. Coumaphos (Baymix, Co-Ral), O,O-diethyl-O-(3-chloro-4-methyl-2H-1-benzopyran-7-yl)phosphorothionate, was effective against a series of cattle worms in 2 mg/kg dose levels for 5 days.[123] Thimet (O,O-diethyl-S-{(ethylthio)methyl}phosphorodithionate was studied for tolerance in chicken embryos,[124] trichlorophon was found effective (7.5-15 mg/kg/day) against Mexican[125] but not African[126] strains of Onchocerca volvulus.

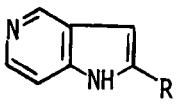
2-(5-Nitro-2-thienyl)-4-substituted aminoquinazolines (XVI) (best R_1,R_2=hydroxyalkyl) were active against Ascaris suum, Syph. obl., and Hymenolepsis Nana in doses of 12.5-300 mg/kg.[127] Imidazo{1,2-α}pyridines (XVII) (best R_1=ROCONH-, R_2= ) were active against



(XVI)



(XVII)



(XVIII)

Trichostrongulus.[128] Some N-substituted arylsulfonylpyrazoles were active against oxyures in mice,[129] and certain azaindoles (XVIII) (most potent R= ) were active against Hoenonchus contortus in sheep at 100 mg/kg levels, single dose, but inactive against other parasites tested.[130]

Diminazene aceturate (phenamidine isothiurate) was found superior against Barbesia gibsoni in dogs.[131] Toxicity of amicarbalide diisothionate, active against Barbesia cavalli, was studied in ponies.[132] Ticarbodine (E.L. 974, α,α,α-trifluoro-2,6-(methyl)-1-piperidine-thiocarboxy-m-toluidide) showed a high degree of efficacy in dogs infected with Acilostoma caninum, Dipilidium caninum, Taenia pisiformis, Toxascaris leonina, Toxacara canis, and Unicaria cenostephala at single doses of 100

mg/kg.[133] β-Alkoxy crotonic acid esters are claimed to be active against pig ascarides and mouse tapeworms.[134] Dithiocarbanilates[135] and 1-halophenyl-3-thiazolylcarbamidoylureas[136] are claimed as anthelminthics.

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Chapter 16. Antiviral and Antitumor Chemotherapy with the Interferon System

Hilton B. Levy, NIAID, National Institutes of Health, Bethesda, Maryland

The interferon system was first described in 1957 by Isaacs and Lindenmann (1) who were studying the interference with the growth of some viruses by prior infection of the host cells with other viruses. They showed that the infected cells produce a protein, interferon, which when applied to other cells of the same species renders the second cells more or less resistant to infection by a variety of viruses. The action of interferon is attributed to as yet only partially understood metabolic alterations induced in the protected cells. The clinical potential of interferon was soon recognized, but a variety of technical problems has prevented application to man in a very serious way. Current efforts in a number of countries are bringing the interferon potential closer to realization. This review will have as its focus developments in interferon research that relate to application, but a number of peripherally related areas will be discussed.

Induction and Production of Interferon. A tissue culture system for the production of mouse interferon of high titer has been recently described (2). It involves the use of a virus-transformed 3T3 cell line stimulated by Newcastle Disease virus. Titers of 30,000 to 40,000 reference units/ml are regularly obtained. By dialysis concentration 10 times this titer can be achieved. This will make possible realistic evaluation of interferon therapy using the mouse as a model.

Much effort has gone into the development of methods for the production of human interferon in diploid cells because of the greater acceptability of such a product in the United States than interferon made in leukocytes (3). Significant increases have been obtained in the titer of human interferon from less than 1,000 units/ml a few years ago to over 30,000 and occasionally 100,000 units/ml (4,5).These high levels have been achieved by proper temporal application of cycloheximide and actinomycin to human cell cultures that have been induced with polyriboinosinic.polyribocytidylic acid (poly rI:poly rC) (4-8). Studies with these inhibitors have indicated that, paradoxically, under conditions where RNA and protein synthesis was inhibited, larger amounts of interferon could be

produced both in rabbits (9,10) and in rabbit (7) and human (5,6) tissue culture than under non-inhibited conditions. The effect is explainable by the hypothesis that under ordinary conditions, after induction of interferon synthesis a new messenger RNA is made that leads to the synthesis of a control protein which blocks the continued translation of interferon messenger RNA. Application of cycloheximide and actinomycin D at suitable times blocks the formation of this control protein and allows the continued synthesis of larger amounts of interferon.

Marked increases in the specific activity of mouse interferon have been possible. Specific activities of up to 1×10^8 units/mg of protein have been achieved (11,12). The use of affinity chromatography on sephadex bound antiinterferon globulin is one of the newer useful techniques. Part of the problem of purification lies in the fact that interferon is such an extremely active molecule that only very small amounts are present even in potent preparations. In addition, when cells are induced to form interferon they form other proteins very similar to interferon in physico-chemical characteristics (11).

A number of quite diverse materials are capable of causing either tissue culture cells or animals to synthesize interferon; vaccines (13); acidic polysaccharides such as phosphomannans (14) and chemically phosphorylated polysaccharides (15); phage double-stranded RNA (16,17); A protein from E. coli also has been found to be an effective interferon inducer (18). None of the aforementioned materials appear to be able to induce titers of interferon in mice as high as those induced by poly rI:rC. However, poly rI:rC appears to be a poor inducer when used systemically in man and other primates (19,20), even though it is effective in human tissue culture. Man's refractoriness may be partly attributable to the presence of high hydrolytic capacity in human serum towards poly rI:rC (21). These other compounds might prove better than poly rI:rC in man.

The extent of response of an animal to an interferon inducer may be related to his physiological state (22). Animals with either induced Friend leukemia or spontaneous AKR leukemia yield much less interferon in response to Newcastle disease virus or poly rI:rC (23) than do normal mice, but these observations are difficult to confirm.

Chemotherapeutic attempts with interferon inducers and interferon:

Animal systems, including tissue culture. The interferon system has been demonstrated to be effective prophylactically and in some cases therapeutically against a number of viruses in animals in studies during the past few years. Interferon itself was effective prophylactically and marginally effective therapeutically versus experimental rabies in rabbits and mice (24,25). Poly rI:rC was even more effective (26-28). Tilorone and poly rI:rC were found useful prophylactically against tick-borne encephalitis in mice (29). Foot and mouth disease in mice and in tissue culture was sensitive to the interferon system (30,31). In mice with West Nile virus the protective prophylactic and therapeutic effect of poly rI:rC was ascribed to an action of interferon on the affected brain rather than just to the suppression of viremia (32). Mice which were too young to be immunologically competent still were protected against pseudorabies virus by statolon (33). Herpes virus hominis was inhibited by poly rI:rC both in tissue culture and in mice (34). Studies with Avian influenza reveal that the virus was sensitive to interferon, poly rI:rC and statolon in tissue culture but in six week old chicks only statolon induced interferon and protected the birds. Tilorone was not effective either in tissue culture or in vitro (35).

Ever since the observations that poly rI:rC had pronounced therapeutic value in herpes keratoconjunctivitis in rabbit eyes (36) this system has provoked strong interest. Much less effect was found in vaccinial keratoconjunctivitis (37). In the owl monkey, double-stranded RNA and tilorone were ineffective in inducing interferon and in protecting against ocular infections, while concentrated preparations of human interferon did prevent infection (38). It has been suggested that local interferon induced by ocular infection with herpes is responsible for recovery from primary infection while local antibody is responsible for prevention of reinfection (39).

The degree of resistance to infection with Semliki forest virus induced by a variety of conditions that stimulate the production of interferon appears to be related to the amount of interferon induced (40).

A number of techniques have been used to increase the titer of interferon produced by animals in response to inducers. Poly-d-lysine forms a complex with poly rI:rC, which complex induces somewhat higher titers of interferon in

mice than does poly rI:rC alone (41). The "antiviral" preparation chlorite oxidized amylose (COAM) (42) which exerts its antiviral effect primarily by means other than the interferon mechanism (43) when given to mice or cats 3 hours before poly rI:rC or Newcastle disease virus strongly augments the interferon production induced (44). COAM also is a potent enhancer of immune reactivity (43-45).

There have been interesting reports of the effect of the interferon system on protozoa (46-49). Poly rI:rC protected mice against Trypanosoma congolense probably because of the immune enhancing action of the drug rather than because of its interferon producing capacity (47). On the other hand it has been shown that poly rI:rC enhances the pathogenicity of T. cruzi in mice (48). Claims have been made that virus inducers of interferon protect mice against Plasmodium berghei by stimulating spleen macrophages (49), although the data presented could be interpreted in other ways.

Interferon (50) and interferon inducers (51) have been shown to inhibit the growth of a variety of tumors, both virus induced, transplanted and spontaneous. New reports expanded this list recently (52-56). In addition poly rI:rC given as a single dose before murine sarcoma virus can actually enhance tumor production by the virus (57,58). The mechanism of the antitumor action of poly rI:rC is complex (59). It can induce interferon, and interferon can exert a strong antitumor action-although the amount of serum interferon induced by poly rI:rC is not necessarily related to the degree of antitumor activity (60); poly rI:rC also enhances immune reactivity of the host (61). Interferon itself under certain conditions also can enhance antibody production in mice (45) and not under others (62). There is also a cytotoxic action of poly rI:rC in mice (63). Interferon has been reported to exert growth inhibitory effects on cells in tissue culture (64). This inhibitory effect applies to normal tissues as well as to tumor cells (22, 65-68). These cellular effects are not associated with death of the cells but rather with a reversible slowing of the growth rate. Thus the concept that interferon is exclusively an antiviral substance is being replaced by the idea that interferon may be concerned with regulatory mechanisms and possibly other cell functions including antibody production (45), specific cytotoxicity induced by lymphocytes (66) and macrophage action (69).

The specific event involved in interferon induction by viruses is still not completely understood. While some

workers contend that the formation of double stranded RNA or RNA-DNA double-stranded hybrids is the inducing event (70,71), at least two pieces of evidence indicate that in certain virus cell systems it is the infecting virion that is responsible. With Newcastle Disease virus it has been shown through the use of metabolic inhibitors that synthesis of the messenger RNA for interferon takes place even when no virus components have been synthesized (72,73). Also using chikungunya virus in chick cells, pretreatment of the cells with interferon blocks formation of all virus components, yet enhances the amount of interferon formed (74). With reovirus, which comes into the cell with a double-stranded RNA genome, interferon is not made until long after virus components have been synthesized (75,76). Suggestive evidence was presented that in still another system, interferon induction was associated temporally with the production of viral RNA polymerase (77).

Differences continue to exist about the effect of alteration of molecular weight of poly rI:poly rC on its antiviral activities and its toxicities. These differences may relate to different methods of preparation of the poly rI:poly rC (78-80).

Therapeutic Trials in Man. Extensive trials in man with synthetic inducers and viral vaccine inducers of interferon as well as with interferon itself have been reported in the Soviet Union. These results have been very encouraging, but confirmation from other countries has not been forthcoming. In a study with 2000 children aged 1 to 7 years, interferon or a placebo was given intranasally, before and during epidemics of respiratory disease, including influenza. A very marked diminution of the incidence and the severity of the disease was found in the group receiving interferon (81), even though the amount of interferon given was small. Therapeutic value of leukocyte interferon in herpetic keratoconjunctivitis in man was found both in the superficial form of the disease and in cases where there was deeper involvement of the cornea and uvea tract (82). Vaccination of volunteers with several strains of influenza vaccine gave interferon levels comparable to those found in natural infection with Hong Kong virus (83). In studies with workers in several factories during an influenza virus epidemic, controlled studies with 3 viral vaccine inducers of interferon had significant protective effects (84-87). In England volunteers received a variety of viruses intranasally and interferon was found regularly in the nasal washings (88). The implication of this study is that intranasal instillation of attenuated viruses might be

valuable in treating virus infections in the naso-pharynx area. In another study carried out in England large amounts of leukocyte interferon (several million units) were found valuable in preventing experimental infections in volunteers (89). Amounts less than a million units were ineffective. The large amounts needed would present a severe problem in use on a large scale at the present time.

The possibility that disease in a patient may influence his ability to produce interferon was suggested by studies in which interferon production in vitro by lymphocytes from normal patients was compared with that from uremic patients. The latter produced much less interferon on stimulation by Newcastle disease virus (90).

Poly rI:rC in experimental rhinovirus prophylaxis in volunteers gave meaningful but not dramatic improvement (91). As with the studies with interferon itself, the results show that the interferon system can be efficacious. With all inducers studied, in animals and in man, repeated stimulation within a few days after the primary stimulation produces less interferon than does the primary stimulation (92). Methods to overcome this hyporesponsive state have not been satisfactorily developed and pose a severe problem in the use of inducers.

These several scattered reports all suggest but do not establish the possible efficacy of the interferon system in man. It is apparent that larger amounts of human interferon or better inducers are needed. While the toxicity of poly rI:rC in man is not high (93,94) the drug is not particularly effective in man (95-97). Efforts during the next few years will probably continue along the development of better inducers of interferon as well as increased production of the interferon molecule itself in tissue culture.

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Chapter 17: HOST MODULATION OF RESISTANCE TO INFECTION AND NEOPLASIA

William Regelson, M. D.
Medical College of Virginia, Virginia Commonwealth University
Richmond, Virginia 23298

The resistance of an animal to infection or cancer is an interrelated problem which involves common pathophysiology that effects adrenal function, immunologic response, interferon induction and phagocytic activity. A better understanding of these patterns of interrelating physiology has given rebirth to major interest in the utilization of non-specific host stimulation as a means of controlling infection and/or tumor induction and growth. Non-specific stimulation of host resistance may be of importance to prophylaxis or treatment of chronic disease as there is now increased recognition of the role of slow or latent viruses in the production of degenerative disease that relates to patterns of immunologic response, neoplasia and aging.[1]

The concept of non-specific host stimulation which is synonymous with "stimulation of phagocytes" is an old concept for modifying or preventing disease. However, in contrast to improving host resistance with specific immunization which has been established since the time of Jenner, the use of non-specific agents for increasing resistance has only recently become separable from homeopathy. Non-specific therapy is no longer dependent on biologicals (e.g. Bacillus Calmette-Guerin vaccine (BCG), mixed bacterial toxins (MBT), etc.), but now has a "stable" of well-defined chemical agents that show structural activity relationships and oral as well as parenteral activity. We now have chemotherapy that can simultaneously provide a rational approach to control of virus, bacterial, fungal and protozoal infection as well as inhibition of tumor induction and growth through stimulation of phagocytosis and modulation of immunologic response.

In relation to the above, the role of stress in chronic and acute pathophysiology has been emphasized ever since steroidal therapy became clinically applicable. However, hormones other than that of steroidal origin can be important in resistance to infection. It has largely been forgotten that before the days of the effective chemotherapeutic control of tuberculosis, Lurie[2] showed that resistance to tuberculosis infection was increased on the administration of thyroid hormone in an experimental rabbit system. In addition, he noted that thyroxin decreased the spontaneous appearance of uterine tumors in these rabbits. Recently, we have found that thyroxin and tri-iodo-thyronine stimulate phagocytosis in mice,[3] and this deserves further investigation with particular reference to the fact that thyrotoxicosis is associated with lymphocyte mobilization which may indicate other potential roles for thyroid hormone via lymphocyte or macrophage response.

Emotional factors can effect viral susceptibility. During the influenza epidemic of 1957-1958, Cluff and Imboden et al.[4,5] did a prospective epidemiologic study to determine what factors altered susceptibility to influenza infection in a healthy population. The only parameters that related to susceptibility were those determined by psychologic testing as there was a significantly greater frequency of influenza in persons previously identified as being psychologically vulnerable. Those vulnerable to influenza fell into a readily classified despondent group overly concerned with illness. Susceptible individuals showed a suggestive increase in both clinically demonstrable and non-demonstrable infection, and in addition their illness was prolonged as compared to the non-psychologically vulnerable. This area of research is pertinent to the recent work of Gross and Calmano[6] who have shown that stressed chickens (socially crowded before pecking order can be established) were more resistant to bacterial infection (E. coli, S. aureus and S. faecalis) but more vulnerable to virus and mycoplasma infection. In contrast, the non-stressed chickens were more susceptible to bacterial infection but showed much more resistance to the sarcoma-producing virus of Marek's disease and to Mycoplasma gallascepticum. Decreasing degrees of social stress increased the resistance of these chickens to virus and mycoplasma.

Pertinent to the above, one could chemotherapeutically modulate this response pattern[7] by administering metyrapone (2 methyl-1,2,di-3, pyridyl-1-propanone) which can selectively inhibit adrenal 11-beta-hydroxylase activity which is needed for cortisol and cortisone synthesis. In addition to metyrapone, DDD (dichloro - diphenyl-dichloro ethane) which produces adrenal cortical atrophy when fed to chickens, decreases the incidence of severe response to mycoplasma, hemmorrhagic enteritis virus and Marek's disease.

Observations of this kind are important in mammalian systems as well, in that Marek's disease is a herpes virus and the emotional or stress related vulnerability of patients to the related herpes simplex and zoster is well known. This is of increasing importance in view of the association of herpes viruses to Burkett lymphoma and epidermal carcinoma, and Jensen[8] has presented data that non-specific or psychologic stress can alter host resistance to Rauscher leukemia virus. Infected mice, subjected to sound or avoidance learning stress developed significantly smaller leukemic spleens than non-stressed infected mice.

The mechanism of action by which adrenal cortical function can moderate resistance or susceptibility to infection may be independent of the action of steroids as anti-inflammatory agents. What might be of concern here is the role of steroids as lymphopenic agents which could, by effecting thymus-derived lymphocytes, block cellular immune reactivity which could produce the pathophysiology of the disease. Because of this, one should be aware of the fact that in its turn virus infection can influence immune response. Mice infected with Gross, Rauscher or Friend leukemia viruses show depressed antibody response and the viruses that depress immune response do not have to be leukemia viruses as the slow or

latent LDH or lymphocytic chorio-meningitis virus (LCM) given to adult mice can produce both depression[9,10] and/or stimulation or "helper" activity to antibody response.[11]

These patterns of interrelationship described above deserve further study, and in this regard, the LDH virus can also increase circulating levels of injected asparaginase which may be an effect of immunologic inhibition.[12] The demonstration that viruses have chronic as well as acute effects and can produce hyperlipemias,[13] nephritis, and chronic central nervous system injury that may relate to cellular immunity and latent virus infection is now providing us with clues that may involve non-specific host stimulation as an approach to the treatment or prevention of chronic degenerative disease.

Of importance to psychologic factors effecting host response is the consideration of the compound cinanserin hydrochloride, 2-[3-(dimethylamino)-propylthio] cinnamanilide hydrochloride. This drug was initially developed by Squibb (SQ10643)[14] as a potent antiserotonin agent. A related structure cis-para-hydroxy cinnamic acid was developed by U. S. Vitamin as an anti-inflammatory agent, and this structure has an old history as it was used clinically as a leukocytosis promoting antitumor, anti-rheumatic agent in 1908.[15] Cinanserin[14] showed useful clinical activity in the treatment of hypermotile bowel syndrome as well as carcinoid syndrome in man. Of importance to this discussion, cinanserin has immunologic blocking activity without marrow suppression![14,16,17] In addition, cinanserin possesses analgesic, local anesthetic properties, antiarrhythmic properties, and was shown to be clinically effective as a psychic energizer in the treatment of manic-depressive psychoses in selected patients.[18] However, what is of interest to us is that cinanserin suppresses the primary and secondary immune response of mice to sheep red blood cells and was more effective than Imuran® in reduction of protein synthesis to phytohemagglutinin in stimulated human lymphocytes.[14] It was, therefore, not surprising to find that cinanserin suppressed the development of experimental allergic aspermatogenesis in the guinea pig and also blocked the development of hyperimmune encephalitis in rats. Of potential importance to clinical transplantation studies, cinanserin significantly prolonged skin graft survival in mice.[14,19]

Clinical trial of cinanserin in our hands showed it to be potentially useful in the treatment of a polyserositis related to lupus in one patient and there have been suggestive reports of its value in rheumatoid arthritis. When given alone or in conjunction with hydroxyurea, cinanserin improves survival time of Fischer rats with a methylcholantherene-induced transplanted mammary tumor,[14] and we have shown it to possess activity against Friend leukemia virus splenomegaly.

Cinanserin has not been available for clinical tests because on chronic administration it increased spontaneous tumor incidence in rats.[14] This is most unfortunate as this agent shows real promise, and cinanserin or related structures should be made available for further evaluation and clinical trial.

Chlorphenesin (3-p-chlorophenoxy-1,2-propanediol) is an agent related to the muscle relaxant mephenesin. Chlorphenesin has been shown to prolong survival in Rauscher virus leukemia, and the L1210 leukemia as well as to produce regression of the established L1210, the Walker 256 carcinosarcoma and the plasma cell tumor MOPC21.[20] In a recent paper, it has been shown to have some clinical value in the treatment of selected epidermoid cancers in man, although it did not have clinical value on limited trial in acute leukemia. Like cinanserin, this compound is of interest because of immunologic effects. It can block penicillin-induced passive cutaneous anaphylaxis in guinea pigs.[21] This immunologic blocking activity is of importance because it is selective depending on the antigen administered, as bovine serum albumin sensitivity is unaffected. Chlorphenesin has no marrow suppressing activity and its mechanism of action may relate to reports of it blocking sensitized leukocyte release of histamine to ragweed antigen,[22] and this may relate to inhibition of cyclic AMP.[21]

Most recently, another agent without marrow suppression which shows immunologic antagonism is oxisuran (2-methylsulfinyl acetyl pyridine). This compound has been reported by Freedman et al.[23] to possess differential inhibition of cell-mediated hypersensitivity. While it suppresses allograft skin rejection in mice, it can do this without suppression of hemolytic antibody response to sheep erythrocytes.[23,24] Current preliminary work indicates that it can suppress tumor growth in dimethyl-benzanthracene treated rats.[25]

Despite clinical opprobrium because of its teratogenic activity, thalidomide, N-(2,6-dioxo-3-piperidyl) phthalimide or 2-(n-phthalimido) glutarimide possesses immunosuppressant[26,27] activity without marrow suppression which has been of value in the treatment of lepromatous leprosy.[28] Clinical study of its antitumor activity showed palliative effects but no significant objective regression. Cyclic imides or related structure have been looked at for antitumor activity and 1-(morpholinomethyl)-4-phthalimido-piperidindione-2,6 (CG,603) has shown activity alone or in conjunction with androgen in dimethyl-benzanthracene induced rat mammary tumors.[29]

Another series of compounds of clinical interest are tilorone hydrochloride, 2,7-bis[2-(diethyl amino)ethoxy] fluorene-9-one, dihydrochloride, and related congeners. Tilorone hydrochloride is an antiviral agent which induces high levels of circulating interferon and is effective both orally and parenterally.[30] We have previously shown that tilorone produces stimulation of the reticuloendothelial system (RES) activity when administered orally,[31] and Adamson demonstrated its antitumor activity against the Walker 256 carcinosarcoma and a reticulum cell sarcoma when injected intraperitoneally.[32] There are now available a large number of congeners related to tilorone whose structural activity relationships have been thoroughly studied in regard to antiviral action.[33]

In relation to the mechanism of action for tilorone, it was similar in its antitumor and antiviral spectrum to the synthetic polynucleotide, poly rI:rC. Tilorone also resembles poly rI:rC in that it is a potent immunologic adjuvant and is like the synthetic polyanion, pyran copolymer, in possessing phagocytic stimulating activity and can prolong skin grafts in mice, but tilorone does not protect against lethal bacterial or cryptococcal infections to the same degree.[34]

Tilorone HCl and five antiviral congeners were studied for reticuloendothelial (RES), antitumor, immunologic adjuvant and in vivo antibacterial activities. From our study, tilorone related compounds are available which show effective activity against Friend virus and allogeneic Ehrlich solid tumor growth. These compounds protected against lethal staphylococcal infection and show great similarity as well as essential differences from pyran and poly rI:rC.

Tilorone and three related congeners were examined for their ability to enhance 19S antibody response to sRBC. All four compounds were potent immunoadjuvants but the most active compound was DMAE-fluorenone followed by DEAP-fluoranthene and DEAE-fluorenone. In regard to mechanism, tilorone has been reported to stimulate thymic dependent lymphocytes[35] and independent lymphocytes.[36] However, immunoadjuvant activity may not be the critical antitumor mechanism as DEAA-fluorene which was the most effective agent against the Friend leukemia virus tumor and the Ehrlich carcinoma showed the least immunoadjuvant activity of the four compounds tested.

In contrast to the oral route, tilorone when administered subcutaneously did not alter RES function, but this could be a function of dose, since 50 mg/kg is the maximum tolerated dose subcutaneously and 250 mg/kg is the maximum tolerated orally. Thus, oral administration may deliver more drug directly to the liver through the portal system inducing previously reported stimulation of hepatic phagocytic response.[37]

DBAP-anthraquinone induced the most marked phagocytic stimulation of the RES, and mice inoculated i.v. with a lethal dose of S. aureus at the time of maximum stimulation showed 100% survival. This may reflect on enhanced vascular clearance as S. aureus has been reported not to require opsonization for phagocytosis. This is further suggested by the lack of protection afforded in other experiments against D. pneumoniae which requires opsonization. This is again a distinct difference from pyran copolymer, which through its phagocytic-stimulating effects protects against D. pneumoniae as well as S. aureus.

In tissue culture, tilorone and related compounds possess cytocidal activity against a variety of cell lines equal to that seen for nitrogen mustard,[37] but paradoxically in vivo no bone marrow toxicity is seen despite the presence of basophilic or Feulgen positive inclusions in macrophages and leukocytes.

In Friend leukemia, tilorone administered daily after virus inoculation was ineffective but did show antitumor action when administered as late as 5-13 days post virus, suggesting that interferon production or the antiviral effect may not be representative of the antitumor mechanism in this virus tumor system. Of interest, doses of tilorone ordinarily tolerated by normal mice produced toxic death in mice hosting the Friend leukemia suggesting a metabolic alteration in mice suffering from FLV infection that relates to drug action.

The most active congener against FLV tumor growth was DEAA-fluorene which like tilorone was also effective in inhibiting leukemic splenomegaly of the established disease. Similar effects were seen for DMAE-fluorenone, and the mechanism whereby these agents inhibit the splenomegaly is not known, but it is not related to interferon effects against the causative Friend virus, as pretreatment 24 hours before virus inoculation at the height of circulating interferon levels was ineffective.

In contrast to the autocthonous Friend leukemia system, tilorone was ineffective in inhibiting the growth of the Ehrlich carcinoma solid tumor, but DEAP-fluoranthene gave a 93% tumor inhibition and DEAA-fluorene showed a similar level of activity.

Although none of these compounds was effective on parenteral subcutaneous administration in inhibiting the L1210 syngeneic leukemia, when tilorone, DMAE-fluorenone and DMAE-xanthone were given in drinking water significant prolongation of survival was seen.[38]

In a Phase I and early Phase II clinical study, tilorone has produced partial responses in two of six patients with melanoma, and one of four breast cases for periods ranging from two to six months.[39] This was seen in patients who were on therapy two weeks or longer. Tilorone does not depress the bone marrow, and on the contrary appears to stimulate platelet production. In our Phase I and Phase II study of tilorone's antitumor action, a rather consistent initial stimulation of the platelet count was seen but following this the count showed a tendency to fall in spite of the fact that the concentration of the drug in tissue or tissue fluids was presumably increasing. Thus, the stimulation of platelet count by tilorone appears to resemble that seen for interferon induction, namely stimulation followed by exhaustion of response on repeated administration.

The toxic effects of tilorone in man are occasional lethargy seen in close association to drug administration, but nausea and vomiting, anorexia, diarrhea, bizarre dreams and insomnia have been seen at upper dosage levels.

In relation to the clinical action of tilorone, we have developed some animal evidence that schedules of administration can influence toxic effects. This is important as Gazdar et al.[40] have shown that this could

be pertinent to paradoxic promotion of tumor growth which has also been seen for pyran and poly I:C. The exploration of various drug schedules is essential.

Further clinical investigations of tilorone and related structures is necessary as these compounds represent a new departure from previous antitumor substances that stimulate host response. Prolongation of skin grafts,[41] and blockade of hyperimmune encephalitis and adjuvant induced arthritis,[36] suggest that these and related structures be studied in transplantation and hyperimmune disease as well.

Most recently a new oral and parenteral interferon inducer has been discovered because of production of basophilic granules in rat lymphocytes similar to that seen with tilorone.[42] This compound BL-20803, is 4-(3-dimethyl aminopropylamino)-1,3-dimethyl-1H-pyrazolo(3,4-b) quinoline dihydrochloride. Of interest in regard to the discovery of BL-20803 interferon induction is that although many congeners of tilorone induce basophilic inclusions in phagocytes or lymphocytes this is not necessarily associated with interferon inducing capability and interferon inducing capacity does not always correlate with successful antiviral activity.[43] In that regard, the antimalarial Atabrine® can induce similar cytoplasmic inclusions in white cells and also has interferon inducing capacity.[43,44]

Dr. Hilton Levy in a previous chapter has discussed some aspects of the interferon inducers, one of which is pyran copolymer (DIVEMA NSC-46015). Pyran readily hydrolyzes to form a poly-carboxylate polyanion which *in vivo* shows a variety of biological activities. It is an inducer of interferon and shows activity against a wide variety of RNA and DNA viruses including Rauscher leukemia, Moloney and polyoma viruses, and encephalomyocarditis virus.[45,46,47,48,49] The level of circulating interferon produced by pyran does not relate to the antiviral effects seen, as protection against meningoencephalitis can be seen one month after single interperitoneal injection[47]; thus it differs from the synthetic polynucleotide poly I:C and tilorone.

Pyran was equally effective in controlling tumor growth in mice inoculated with Rauscher and polyoma tumor virus that had been severely immunosuppressed by thymectomy and treatment with antilymphocyte serum.[49] These results would suggest that pyran might exert its antitumor or antitumor virus effect by reversing virus immunosuppression or through a mechanism other than that of either the humoral or cellular immune response. Hirsch's data suggests that pyran could conceivably maintain the effectiveness of thymus derived lymphocytes in the absence of an active thymus, and this mechanism has been shown to be operant for certain polynucleotide immunoadjuvants. Polyanions, including native heparin, have also been shown to alter steroid metabolism and lymphocyte distribution.[54]

In addition to effects on spontaneous cancers, treatment of hamsters with pyran has decreased the incidence of dimethylbenzathracene induced cheek pouch tumors during the induction period, but once tumors

appear, pyran appears to accelerate their growth.[50] This is pertinent to the work of Gazdar et al.[40] who has reported that with certain regimens, pyran can enhance the formation of murine sarcoma virus tumors in certain strains of mice but not in other strains.

Pyran is not directly cytotoxic[37] but has been shown to be effective against a variety of allogeneic and syngeneic transplanted tumors.[51-55] As an example, treatment of mice daily for 11 days following inoculation of Lewis lung carcinoma is as effective in antitumor programs as treatment with the reference compound, cyclophosphamide.[56]

Recent studies reported by Chirigos[57] have demonstrated the ability of pyran to maintain remission of a lymphosarcoma that has been induced with standard chemotherapeutic agents such as BCNU. Of interest, despite apparent cures in these animals, they may be once more susceptible to growth of this tumor upon re-inoculation. These studies are exciting because they suggest that pyran could have a clinically effective adjuvant action when the tumor burden has been adequately reduced by surgery, radiation or standard chemotherapy. In relation to standard chemotherapy, one cannot use pyran and methotrexate or cytoxan simultaneously as these two drugs abrogate the pyran effect indicating the probable necessity for the presence of host immunologic factors. This is pertinent because pyran possesses immunoadjuvant activity as relates to production of 19S antibody against sheep erythrocytes,[58,59,60] and as is also true for tilorone, data in our laboratory indicates that pyran can prolong skin graft survival.[58] These observations are also supported by the work of Kapusta and Mendelson[61] who show that pyran could block adjuvant-induced arthritis in rats. How this relates to antitumor activity is not clear, but it suggests that pyran may increase production of cytotoxic antibody or deblocking factors which could also result in tumor rejection despite inhibition of cellular immune response. Alternatively, this could relate to increased macrophage killing capacity.

Of major importance, pyran has been shown to increase the effectiveness of a killed virus vaccine for foot-and-mouth disease virus.[62] The combined inoculation of vaccine and pyran was much more effective than administration of either agent alone. This activity was seen for pyran at doses which were completely non-toxic in the mouse system, and pyran effectively enhanced the activity of the vaccine at concentrations too low for pyran to exert any antiviral effect of its own. These effects are seen in the absence of any evidence of increased circulating antibody or cellular immune response against this particular virus.

In mice, pyran has been shown to cause hepatosplenomegaly, thymic atrophy, inhibition of hepatic microsomal mixed functional oxidase enzymes, weight loss, anemia and leukocytosis.[63,64,65] Of interest to its effects on increasing resistance to cryptococcal, staphylococcal and pneumococcal infection, it can paradoxically sensitize mice to the lethal effects of gram-negative endotoxin.[66] Pyran can also cause elevations in serum glutamic oxalotransaminase as well as serum acid phosphatase.

Pyran has been tested clinically by us for antitumor activity in 67 advanced cancer patients.[67,68] Its major toxic side effects were thrombocytopenia and pyrexia, and hypotension.[68] In high dose, it caused an acute hemolytic-uremic-like syndrome in certain patients with Dawson's encephalitis.

Of clinical importance, studies conducted in conjunction with Hercules, Inc., Wilmington, Delaware, have shown that the toxicity reported for pyran can be significantly decreased,[69] while the antitumor activity of pyran can be maintained against the Lewis lung carcinoma and other selected tumors.[70] Toxicity for pyran copolymer appears, to a large extent, to relate to molecular weight. On fractionation, the higher the molecular weight the greater the degree of toxicity as measured by endotoxin sensitization, weight loss, anemia, hepatosplenomegaly, SGOT elevation and leukocytosis. Of importance, the biphasic effect of the clinical parent compound (NSC46015) on phagocytosis (inhibition of phagocytosis followed by stimulation) may relate to the fact that the parent clinical compound is non-uniform in its molecular weight distribution. The higher molecular weight fractions of pyran block phagocytic response while lower molecular weight fractions show a pure phagocytic stimulatory activity. The biphasic effect on phagocytic response thus appears to represent a reaction to both high and low molecular weight fractions. Thus, separation of this or other polymers by molecular weight into more uniform fractions could select for more specific biologic activity in relation to host response.

The antitumor activity of pyran is essentially independent of molecular weight as both high and low molecular weight fractions possess significant antitumor activity and one is now able to preserve potent antitumor activity without associated toxicity.

Molecular weight differences effecting biologic activity have also been seen for polynucleotides[64] and for ethylene maleic anhydride copolymers[54] which also possess distinctive antiserum complement activity related to molecular weight fractions studied.

Pyran is also useful as an agent for removing plutonium from its storage in liver and other tissue.[71] The mechanism of action for this is not clear, but similar effects have been reported for other agents which effect reticuloendothelial function and induce interferon.

Because of our ability to control structure and molecular weight and thus modify toxicity or biologic activity, pyran possesses inherent advantages over BCG and Corynebacterium parvum which have been shown to be effective in similar experimental conditions against a number of transplanted tumors. In addition, unlike the polynucleotides, pyran is not readily biodegradable although the bulk of the clinical compound is gone from liver and spleen in three months. With effective lower molecular weight material more rapid excretion might be found. One might expect newer pyrans or related polyanions to be an important addition to newly emerging groups of immunoadjuvants useful in cancer chemotherapy or in

control of infectious disease, or metal poisoning. Similarly, tilorone and related congeners possess equal advantages, and in addition can be orally as well as parenterally active. It will be of interest to see the results of further Phase II study with tilorone as an antitumor or antiviral agent. One must remember that tilorone is the first of many related structures to come into clinical trial, the next being DEAE-fluorene.

All these agents discussed here should have adequate clinical test. In tumor studies, one would guess that their main role might be their place as adjuvants to other forms of chemotherapy or to ablative surgery and radiotherapy although they do have primary antitumor activity on their own. A major place for these agents as adjuvants might relate to their use in conjunction with the development of tumor or tumor virus vaccines. The work of Campbell and Richmond with foot-and-mouth disease[63] is a case in point for further work with vaccines to see if these agents which alter RES function could be useful for more effective specific as well as non-specific therapy.

An understanding of mechanism of action for this diverse group of compounds should result in the clinical availability of non-marrow suppressing agents which can be useful in organ transplantation or hyperimmune disease. With some confidence, one can say that this decade will see the use of host modulators in the treatment of both specific and non-specific disease.

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Section IV - Metabolic Diseases and Endocrine Function

Editor: Walter T. Moreland, Pfizer, Inc., Groton, Connecticut

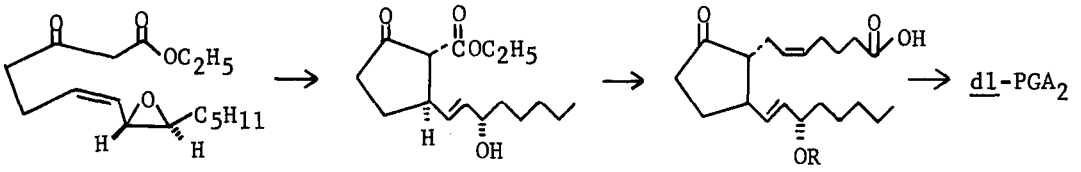
Chapter 18. Prostaglandins and Related Compounds

Richard A. Mueller, G. D. Searle and Co., Chicago, Illinois

The field of prostaglandins has continued its rapid growth; therefore this review will emphasize chemical developments since the last article in this series.[1] The selection of biological topics is arbitrary with an emphasis on the utility of derivatives of the natural materials. In general, very little on structure-activity relationships has been published.

Several books were published on the prostaglandins last year[2-4] and chemical reviews are available.[1,2,5] Specialized reviews on selected biological areas are referred to in the text. The Worcester Foundation publishes, free of charge, a bimonthly bibliography of reviews, books, meeting announcements and original articles.[6] The proceedings of an International Conference on Prostaglandins held in Vienna in 1972 is available.[7]

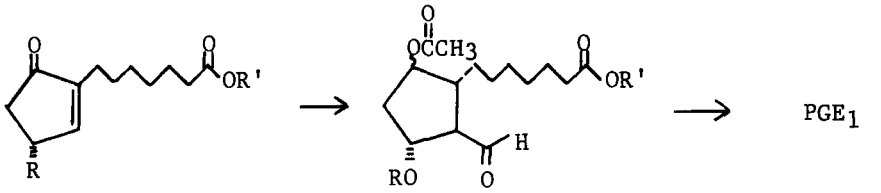
Synthesis of natural prostaglandins and their analogs. A novel direct synthesis of PGA_2 has been achieved by workers at Roussel.[8] The first step



1 2 3

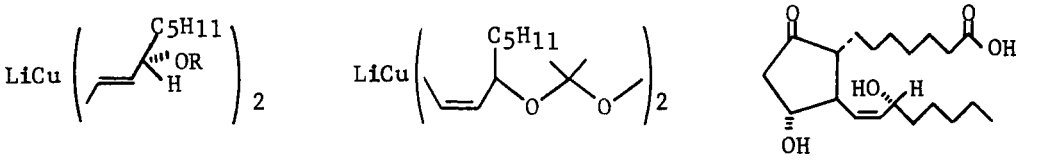
involved generation of 2 by cyclization of the anion of the enamine of 1. The relative stereochemistry at positions 12 and 15 was controlled by proper choice of a cis double bond and trans epoxide. Compound 2 was converted to 3 (R = H), (dl-10,11-dihydro-PGA_2 / dl-11-deoxy-PGE_2), by an alkylation-decarboxylation sequence. Bromination-dehydrobromination of the enol ether of 3 (R = THP) yielded dl-PGA_2.

Two groups at the Syntex Research Institute reported the synthesis of PGE_1. Both groups utilize 4 as starting material. The first synthesis[9] involves 1,4 addition of a vinyl group to 4 (R = OTHP, R' = CH_3) and the conversion of this material to the aldehyde 5 (R = THP, R' = CH_3). 5 is then converted to PGE_1 in six steps. The overall yield is 3.7%.



4 5

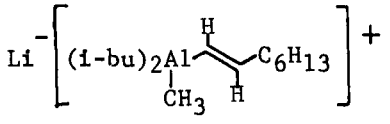
The second approach[10] involves reaction of 4 (R = OH, R' = CH_3) with methyl-isopropenyl ether and phosphorus oxychloride to give 4 (R = $OC(CH_3)_2OCH_3$, R' = CH_3). This substance was treated with the S isomer of lithium divinyl cuprate 6 (R = $C(CH_3)_2OCH_3$) and the protecting groups were removed with acetic acid-water to give PGE_1 methyl ester. Removal of the methyl ester enzymatically using a pancreatic lipase preparation afforded (-)PGE_1. The same group, in a later communication,[11] reported the synthesis of 12,13-cis-PGE_1, 8, using the analogous cis lithium divinyl cuprate, 7. A unique feature of this synthesis is that 1,4 addition to 4 (R = $OC(CH_3)_2OCH_3$, R' = CH_3) of 7 gives only 8. The R isomer of the copper reagent adds stereoselectively to the β face of the enone and the S isomer adds only to the α face of the same substrate. These results were corroborated using the resolved lithium copper reagents. No other isomers were detected.



6 7 8

Sih and coworkers reported[12] the synthesis of PGE_1 as an extension of their earlier work in the 15-deoxy series.[30] Ketone 4 (R = OTHP, R' = Et) was the substrate for a 1,4 addition of 3(S)-(α-ethoxy)ethoxy-1-lithio-1-trans-octene in the presence of tri-n-butylphosphine-copper(I) iodide complex. Acid catalyzed removal of the protecting groups at 11 and 15 and hydrolysis of the ester with bakers' yeast yielded (-)PGE_1. Enantiomeric 11,15-epi-PGE_1 and ent-15-epi-PGE_1 were also obtained.

Workers at Lederle have reported two methods of accomplishing a 1,4 addition reaction. The first consists of the reaction of the lithium alkyl vinyl aluminate 9 with 4 (R = H, R' = C_2H_5) at room temperature to yield (±)-11,15-bis-deoxy-PGE_1 after base hydrolysis.[34] Only the vinyl radical is transferred. The second method involves the tri-n-butylphosphine copper iodide complex promoted 1,4 addition of a Grignard

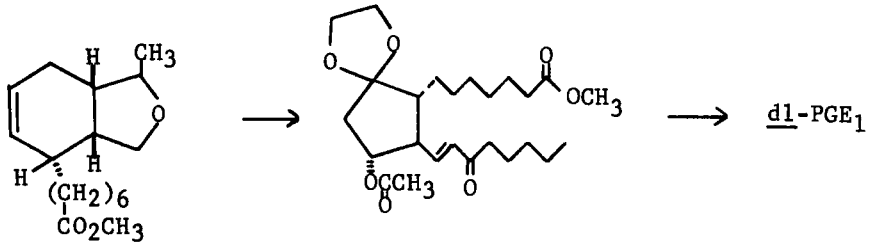


9

reagent.[35] The eventual products are (±)-11-deoxy-13,14-dihydro-PGE_1 and its isomers.

Pappo and Collins at Searle have reported[37] the synthesis of (±)-15-deoxy-PGE_1, its stereoisomers and (±)-11-epi-12,13-dehydro-PGE_1 methyl ester. Their synthesis was accomplished via a 1,4 addition either to 4 (R = OH, R' = CH_3), 4 (R = OTHP, R' = CH_3) or 4 (R = H, R' = H) using organometallic reagents derived from copper, aluminum or gallium. The resolution of 4 (R = OH) has been disclosed.[38]

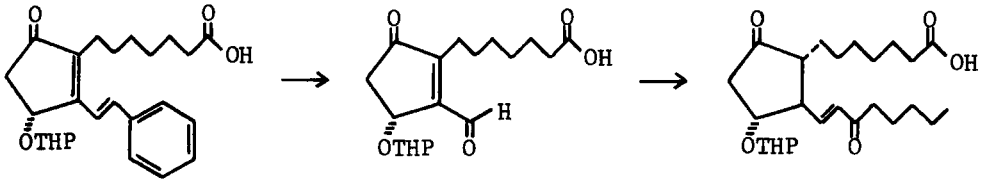
In an extension of their earlier work, Taub and coworkers reacted methyl cis-8,10-undecadienoate with β-angelica lactone to yield 10.[13] The synthesis proceeds in thirteen steps to give 11 which was converted to dl-PGE_1.



10 11

Kojima and Sakai have disclosed a synthesis of PGE_1.[14] Although nineteen steps, the stereochemistry of the ring was established at an early stage.

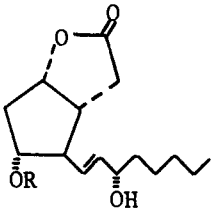
Miyano and coworkers have published a new synthesis of (-)PGE_1.[16] Cleavage of 12, whose resolution has been reported,[15] gave 13 which was then subjected to chromous ion reduction followed by reaction with the appropriate Wittig reagent to give 14. Reduction of the 15-keto group of 14 with lithium tetrahydrolimonyl thexyl borohydride and acid hydrolysis gave (-)PGE_1.



12 13 14

Corey has reported on several extensions of his prostaglandin synthesis,[1] the first of which involves the stereoselective generation of the 15(S)hydroxyl in 15 (R = CO-Ph-Ph).[17] Three requirements must be met for stereospecific reduction of 16 (R = CO-Ph-Ph); a trans coplanar relation-

ship of the hydrogen at 12 and 13, a single S-cis enone conformation and steric approach control of hydride transfer to the 15 ketone. From molecular models it was evident that the p-phenylbenzoate group on the C-11 hydroxyl fulfilled the first two requirements through van der Waal contact. Effective steric control in the hydride reduction was provided by lithium tetrahydrolimonyl borohydride. Further consideration suggested that the p-phenyl-phenyl urethane would provide more efficient Π bond interaction than the p-phenylbenzoate due to a reduction of the steric effects of the hydrogen on C-11. Experimentally this proved to be the case and reduction



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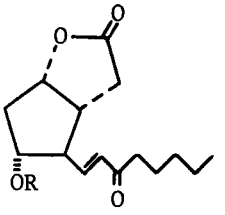
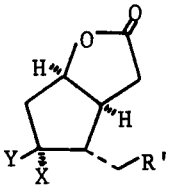


16

of 16 (R = CONH-Ph-Ph) yields 15 with a 15 R/S ratio of 92:8. Removal of the urethane group was accomplished with refluxing lithium hydroxide. Additional publications introduce the t-butyldimethylsilyl moiety as a protecting group for hydroxyl functions[19] and the application of organometallic reagents to model systems for prostaglandin synthesis.[50-52]

A joint communication from Pfizer and Harvard[20] disclosed a variant in which (-)PGE_1 and $PGF_{1\alpha}$ were synthesized via the intermediate 19 (R = THP) by adding the carboxyl-containing seven carbon sidechain first.

The synthesis of ent-11,15-bis-epi-PGE_2, 18, has been reported.[18] The previously developed method of inverting the configuration at C-11 in 17 (X = OTs, Y = H, R = OCH_2Ph) with tetra-n-butyl-ammonium formate was employed to form, after base hydrolysis, 17 (X = H, Y = OH, R' = OCH_2Ph). This intermediate was carried through the standard sequence of reactions to give 18. This material has 50% of the activity of PGE_2 on the isolated rat uterus and inhibits the activity of the natural hormone if added to this smooth muscle preparation prior to PGE_2.



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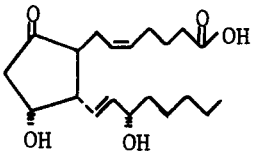

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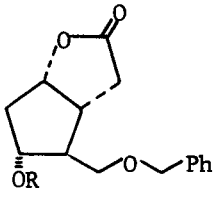


19

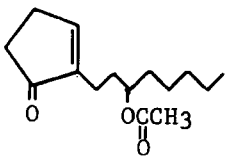
The group at Alza reported the synthesis of 11-epi-PGE_2 and 11-epi-$PGF_{2\alpha}$.[21] The procedure involved was essentially that discussed earlier[18] except that a Moffet oxidation was used for the oxidation of the 9-hydroxy

function to a ketone. The use of a polymer bound diimide for a more efficient oxidation is reported in a later paper.[22] 11-epi-$PGF_{2\alpha}$ was found to be 50% as active as $PGF_{2\alpha}$ on the isolated rat uterus and in vivo for the induction of abortion in the rat. 11-epi PGE_2 had only 12% of the activity of PGE_2 in the same system. No inhibition of the natural product was noted with either isomeric compound. The authors draw the conclusion that the configuration of the 11-hydroxyl is relatively unimportant for this activity in the F series.

A synthesis of ent-11,15-bis-epi-PGE_2 by variation of the Corey method has been disclosed.[25] The cleavage of the methoxy moiety with boron tribromide was successfully accomplished on 17 (X = H, Y = OCO-Ph-Ph, R' = OCH_3).[26] Standard procedures were used to complete this synthesis.[27]

Two new approaches to the synthesis of dl-16 (R = THP) have been described.[23,24] A bicyclic starting material is used in each case to ultimately fix the stereochemistry of the hydroxyl substituents at C-9 and C-11. Both methods involve more steps than Corey's original procedure.

Caton and coworkers reported[28] the reaction of the 1-hydroxy analog of 4 (R = H) with acetone cyanohydrin followed by reduction of the β-cyanoketone to the corresponding 9-hydroxy aldehyde which was then further elaborated to a mixture of isomers of 11-deoxy-PGF_1. A similar sequence of reactions based on enone 20 gave the 9-deoxy prostaglandin isomers.[29]



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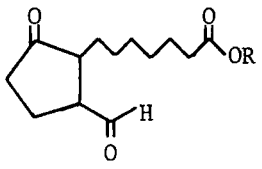
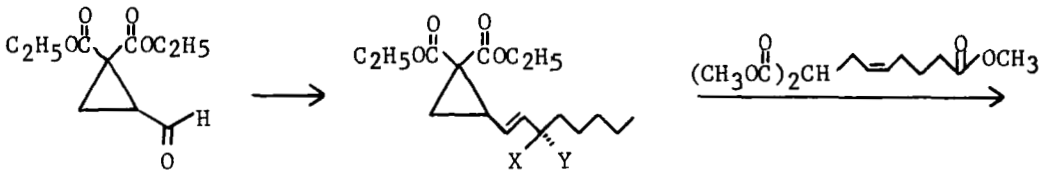


21

Bagli and Bogri have published[31] a complete paper on their photoannelation of 4 (R = H, R' = CH_3) reported earlier. In a second communication[32] these authors reported base catalyzed addition of nitromethane to 4 (R = H, R' = CH_3) followed by sodium in methanol reduction of the product to give the keto aldehyde 21 (R = CH_3). Elaboration of this intermediate gave dl-11-deoxy PGE_1 methyl ester. An alternative synthesis based on the same intermediate yielded (±)-15-epi-11-deoxy PGE_1. The corresponding E_2 series compounds 25 (X = H, Y = OH) are made starting with the appropriate 5,6-cis double bond analog of 4. A later publication[33] discloses a very short synthesis of (±)-11-deoxy-PGE_2, 25 (X = R = H, Y = OH) and (±)-15-epi-11-deoxy-PGE_2, 25 (X = OH, R = Y = H) outlined below.



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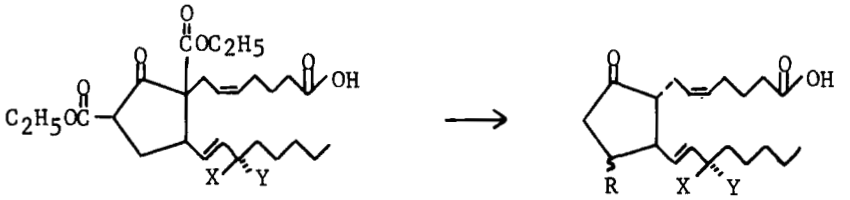
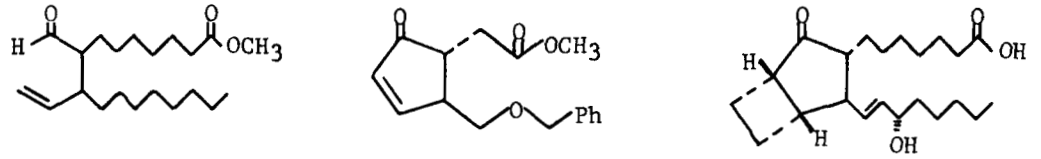


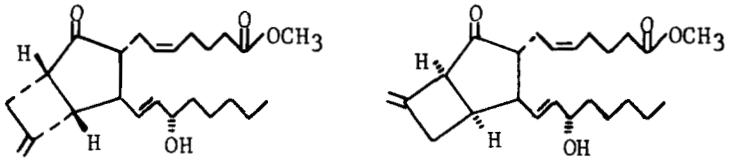
24 25

Sakai and coworkers in Japan have synthesized (±)-11,15-deoxydihdro-PGE_1 methyl ester.[36] Two mild methods of ring closure of 26 were developed: $SnCl_4$ in nitromethane or tris triphenylphosphine chlororhodium in chloroform or acetonitrile. The reaction proceeds at room temperature using either set of reaction conditions.

Crabbé and coworkers prepared (±)-10-α-hydroxy-11-deoxy-PGE_2 and $PGF_{2\alpha}$ from Corey's lactone 19 by a variation of their previously reported methods.[39] The photochemical addition of ethylene to a Corey intermediate 27 afforded a product which was subjected to the standard reaction sequence to give 28. The addition of allene to PGA_2 methyl ester was also disclosed,[40] ultimately yielding 29 and 30.



26 27 28



29 30

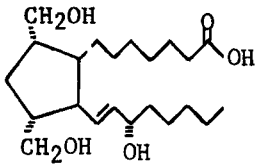
The coral, Plexaura homomalla, continues to provide a good source of starting material for the synthesis of prostaglandins and prostaglandin analogs. Spraggins has disclosed[41] spectral data on the allylic rearrangement products of PGA_2 and PGA_2 methyl ester. The compounds have "slight pressor activity in the rat". Some fifteen compounds derived from Michael

addition of various nucleophiles to the 10,11-double bond of 15-acetoxy PGA_2 methyl ester or its epimer have been reported.[42] One of them, 25 (R = SCH_3, X = H, Y = OH) was converted into PGA_2 in 80% yield by treatment with methyl iodide to form the methiodide, followed by sodium bicarbonate promoted β-elimination.

Katsube and coworkers have reported[43] the synthesis of several isomers of the PGF related compounds, 31, by an extension of methods and the use of intermediates reported previously. The same compounds were made by a group at Syntex who reported 31 to be "weakly active in smooth muscle relaxation assays".[44]

The group at Upjohn has reported two new reactions of the prostaglandins. The first is the simultaneous reduction, resolution and hydrolysis of (±) PGE_2 methyl ester and (±) PGE_1 methyl ester to yield 10% of the natural $PGF_{2\alpha}$ and $PGF_{1\alpha}$, respectively,[45] with bakers' yeast. Also the conversion of $PGF_{2\alpha}$ methyl ester into PGE_2 methyl ester in 35-40% yield[46] was accomplished by selective silylation of the 11- and 15- hydroxyl groups of the F series compound with N-trimethylsilyldiethylamine followed by oxidation of the free 9-hydroxyl with Collins reagent and acid hydrolysis.

The group at Wisconsin has published[47] a complete paper on the biosynthesis and structure determination of 9α,15-(S)-dihydroxy-11-oxo-13-trans-prostenoic acid, 32, which they have named PGD_1. Spectral and physical data are included.



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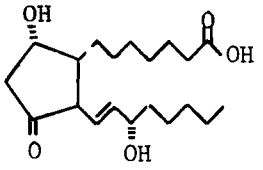

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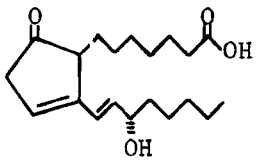


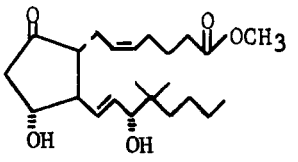
33

Jones has published a full paper[48] on the structure determination of the product of the rearrangement of PGA_1 in cat blood, which he has named PGC_1, 33. He concluded that this is an enzymatic process and the product is responsible for the biphasic response previously recorded when PGA_1 or PGA_2 is injected into animals.[49] The data are consistent with that previously reported for the methyl ester of 33.

^{13}C NMR studies on prostaglandins of the 1 and 2 series have been published.[53] Fried and associates at Chicago have disclosed the details of their PGE_1 synthesis reported last year.[82]

Biological and Clinical Aspects of Prostaglandins. An outstanding development in the fertility area was the introduction of Prostin E_2^R and Prostin F_2 alpha^R by the Upjohn Co. in England and Sweden for the induction of labor and abortion. Several reviews and an annotated bibliography to the clinical reports are available.[54-57] 15(S)-methyl-PGE_2 methyl ester, 35, whose syn-

thesis was described last year, has been found to be 100-500 times as effective as PGE_2 in humans.[58] This greater potency is reported to be partly due to increased intrinsic activity in addition to the lengthened metabolic half-life. dl-Bis-ω-homo $PGF_{2\alpha}$ is five times as potent s.c. and twenty times as active orally as $PGF_{2\alpha}$ in hamsters,[59] and a complete endocrinological profile has been published.[60]



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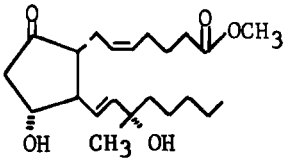

35

Aiken showed that indomethacin and aspirin reduced uterine motility and prolonged parturition in the rat, which resulted in an increase in still-births and the amount of maternal bleeding.[61] On the basis of these results he warns against the use of these drugs in the later stages of pregnancy. A group at I.C.I. reports that indomethacin, aspirin and fenclozic acid delay delivery by 24, 16 and 29 hours respectively and produce an increase in stillbirths.[62] Cortisone acetate had no effect. If $PGF_{2\alpha}$ is given concurrently with the anti-inflammatory drugs, the delivery process is normal. Chronoperiodicity in the induction of abortion with PGF_2 has been observed.[63] Intra-amniotic administration at 6:00 pm leads to the desired effect in a mean interval of 10.4 vs. 26.2 hours when therapy is initiated at other times in the day.

The cardiovascular effects of the prostaglandins have been reviewed[64] and a hypothesis on their involvement in the maintenance of normal blood pressures via the renal system has been published.[65] A report on clinical trials of PGA_1 with sixty hypertensive patients with no untoward effects has been disclosed.[66] Structure-activity relationships with respect to pressure responses in the dog[67] and isolated perfused pancreas preparation[68] have been reported on a number of natural prostaglandins and their analogs.

Three independent reports on the beneficial effects of PGE_1 on stored platelets have appeared.[69-71] Separation of platelets from whole blood is facilitated and there are no side effects when they are administered to humans. Sickle-cell crisis *in vitro* can be induced by PGE_2 and reversed with urea.[72] This is another possible contra-indication to the use of this material to induce labor or abortion in patients who are susceptible to this disease.

Robert presented the antisecretory properties of 16,16-dimethyl PGE_2 methyl ester, 34, and 15(S)methyl PGE_2 methyl ester, 35,[73] when administered I.V. to the dog. 34 is 100 times more potent (ED_{50} = 0.1 μg/kg) than PGE_2. At its oral ED_{50} (10 μg/kg) no diarrhea in induced. The therapeutic effect is prolonged due to metabolic inhibition. Compound 35 is intermediate in potency between PGE_2 and 34; however the therapeutic ratio with respect to

diarrhea and vomiting is similar to that of the natural hormone. 15(R)-methyl PGE_2 methyl ester is reported to be an effective antisecretory drug in the human at a 200 μg dose and to lack gastrointestinal side effects.[74] A short review has appeared on prostaglandins in the antisecretory area.[75]

Rosenthal and coworkers have reported further data on the bronchodilation or constriction effects of the natural prostaglandins and a few analogs.[76] An interesting result is that $PGF_{2\beta}$ is bronchodilating like PGE_2.[77] When administered as an aerosol, the materials have no cardiovascular side effects.[78] The animal work has been confirmed in humans in the case of PGE_2 and $PGF_{2\alpha}$.[79] A warning has been published against the use of $PGF_{2\alpha}$ to induce abortion in women with a history of bronchial distress.[80] An asthmatic human is 10^4 times as sensitive to $PGF_{2\alpha}$ as a normal individual; this effect is not markedly antagonized by PGE_2.[81]

The relationship of prostaglandins to non-steroidal anti-inflammatory agents is covered in chapter 22. Reviews on the role of prostaglandins in cutaneous biology are available.[84-86] The clearing of the symptoms of essential fatty acid deficiency in rats by topical application of PGE_2 has been reported[83] as well as the topical effects of polyphloretin phosphate against PGE_1 induced wheal in the human.[84] The release of prostaglandin during experimental uveitis in the rabbit[87] and during human eye inflammation[88] has been demonstrated. Ocular hypertension following I.V. infusion of PGE_1 has been shown.[89] The mechanism of transfer across the blood-eye barriers appears to be an active transport process[90] and the same phenomenon seems to apply to other tissues in vitro.[91] A review on prostaglandins and the eye has been published.[92]

Kuehl and coworkers at Merck have isolated a receptor site preparation from adipose tissue and have determined the bonding constants of various prostaglandins.[93,94] These constants correlate with cAMP formation and progesterone production induced in the mouse ovary by the same materials. An extension of this work provides a relationship between F series prostaglandins and the formation of cGMP.[95] This data led to the hypothesis that E series compounds act through cAMP and the F series act analogously via cGMP. Normal physiological equilibrium is maintained via the interaction of opposing forces. In general the sequence of events is hormone → prostaglandin synthesis → cyclic nucleotide synthesis → physiological effect, with the prostaglandin synthetase located on the outside and adenylate cyclase on the interior of the cell.

The role of prostaglandins in sympathetic nerve transmission is becoming better defined. Nerve stimulation increases the synthesis of E series prostaglandins which in turn inhibit the secretion of norepinephrine.[96, 97] This results in a negative feedback control or modulation which is supplementary to the α-adrenergic pathway.[98] A review of this subject is available.[99]

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Chapter 19. Atherosclerosis

Thomas R. Blohm, Merrell-National Laboratories,
Division of Richardson-Merrell Inc., Cincinnati, Ohio

Introduction - Recognition of the importance of atherosclerosis as a cause of morbidity and mortality in industrialized countries has resulted in a tremendous development of research directed at understanding and attacking this disease. Because it is impossible to provide a comprehensive technical review within the space available, topics have been selected for discussion on the basis of this author's evaluation of their importance in the current research picture. Some important topics, e.g. lipoproteins, have been omitted this year in the belief that last year's discussion can serve as a guide to current literature.

Pathology and Pathogenesis - Human atherosclerotic lesions which cause clinically manifest disease are complex in structure and take several decades to develop. Simpler lesions can be detected earlier in life, but their relationship to the "mature" lesion has been controversial. This and related problems have been reviewed[1] by the NHLI Task Force on Arteriosclerosis. Their conclusions with regard to pathogenesis are based largely on data obtained in the International Atherosclerosis Project, in which populations with widely differing incidences of atherosclerosis were studied for occurrence of lesion types as a function of age. Three lesion types can be distinguished, that develop in the order: fatty streak, fibrous plaque and complicated lesion. In the early period of fatty streak development, there were no differences in incidence between populations; divergence between populations developed chiefly with the appearance of fibrous plaques. It was concluded that the fatty streak is probably the precursor of the fibrous plaque, but that not all fatty streaks undergo this conversion, in fact, some must undergo regression, with ultimate disappearance (see discussion under Lesion Regression). Since there is little evidence for reversibility of fibrous plaques, the fatty streak-to-fibrous plaque conversion emerges as a critical event in atherogenesis. Identification of the factors which determine different fates for fatty streaks in different populations is an important objective for future research. Differences are known to exist among food fats in their potential for producing fibrotic lesions when fed with cholesterol to experimental animals[2] and McCully has proposed[3] that methionine, derived from animal protein is implicated in the formation of fibrous plaques. This proposal is based on the observation that homocysteinemia has this effect; homocysteine is derived in vivo from methionine. It has been noted previously[4] that much of the epidemiologic evidence which implicates dietary animal fat as an atherogenic factor could also be interpreted to indict animal protein. Hyperlipidemia remains a prime candidate for the role of stimulus for the fibrotic reaction[1], as well as for lipid deposition.

Fibrosis within an arterial lesion can occur only through the intervention of collagen-producing cells. Classically, this function has been

assigned to fibrocytes, but now it has been shown[5] that arterial smooth muscle cells can produce collagen and the other macromolecular components of connective tissue. Migration of smooth muscle cells to the subendothelial space has been recognized for some time as a major contributing factor to intimal thickening, which occurs early in the formation of all types of lesion. Intimal thickening occurs alone in "mechanical stress" areas in young individuals, and has been proposed as the initial lesion of atherosclerosis[1]. This lesion has been produced by mechanical removal of the endothelium in rabbits[6] and monkeys[7]. Participation of smooth muscle cells has been documented[7], and a fibrotic evolution of the lesion demonstrated. Following removal of arterial endothelium in vivo, the denuded intimal surface becomes covered with a layer of platelets, which is gradually replaced by new endothelium[7] (concomitant with smooth muscle cell migration). If the endothelial removal is repeated, platelets now form aggregates (white thrombi) on the surface[8]. It has been reported that repeated injection of ADP (platelet aggregating agent) into the aorta of a rabbit through a catheter left in place for several weeks resulted in appearance of a protrusive atheroma at the point at which the catheter tip touched the artery wall[9]. The importance of this single observation is that an occlusive, lipid-containing lesion was produced without elevation of blood lipids.

The foam cell component of the atherosclerotic lesion is space-occupying, and probably antecedent to degenerative changes seen in complicated lesions. The origins of these cells remain in doubt, although it is established that at least some of them are modified smooth muscle cells. Several important publications have appeared on this subject[10-13].

Fibrin-like material is found in atherosclerotic plaques, and has been cited as evidence for the thrombogenic theory of atherogenesis[14]. Evidence has now been obtained that this material may be a complex of fibrinogen with fibrinopeptides, and therefore not thrombus-derived[15]. The suggestion that it may be cryoprofibrin[15] is strengthened by the finding of elevated levels of circulating heparin-precipitable fibrinogen in atherosclerotic subjects[16].

Lesion Regression - The question of reversibility of the lesions of atherosclerosis has plagued investigators since the disease was defined as a pathologic entity. As knowledge of lesion composition and structure increases, it becomes obvious that what seemed to be answers to this question a few years ago can no longer be so regarded. Certain measurable changes in lesion composition and gross appearance have been observed in man and animals under changing conditions, especially nutritional, which are often reductions in lesion lipid content. In early fatty streak lesions, this can probably be regarded as regression, but it is not certain that reduction of lipid content represents regression in any practical sense in more advanced lesions[1]. Clarkson and coworkers have studied several parameters of lesion change following cessation of cholesterol feeding to the atherosclerosis-susceptible White Carneau pigeon[17-19]. The data were interpreted as following the transition from fatty streaks, containing labile cholesterol deposits (droplets), to a fibrotic lesion

containing a less mobile deposit (crystalline cholesterol) firmly bound in connective tissue; it is not known whether the latter lesion can be caused to regress. At this time it appears to progress despite removal of the dietary atherogenic stimulus, although the picture may be confused by the propensity of the particular animal used to develop spontaneous lesions even in the absence of dietary cholesterol.

Lesion regression has been investigated in rhesus monkeys, but because of the time required to produce advanced lesions in this species, has been limited to fatty streaks[13,20], and uncomplicated lesions with a fibrous cap[21,22]. In the fatty streak lesions (aorta) stainable lipid changed from a predominantly intracellular location to a predominantly extracellular one, and finally disappeared. Essentially normal architecture of the artery wall was restored. In the coronary artery, with a greater fibrous component, there were definite decreases in lesion size and encroachment on the vessel lumen[21], accompanied by a major loss of chemically determined cholesterol and cholesterol esters[22]. In these lesions, unlike those in the pigeon, cholesterol esters persisted as the lipid residue, rather than free cholesterol[22]. The fibrous component of "regressed" lesions in the rhesus monkey appears condensed, rather than removed[22,23].

Reduction in size of complicated lesions in the dog has been reported[23] following surgical diversion of bile to the lower intestine with return to normal diet[24]. Administration of D-thyroxine and normal diet was less effective[23].

Animal Models - Clarkson has reviewed[25] animal models, and documented the trend toward use of primates, which in general exhibit metabolic and pathologic characteristics closest to man. Different species can be used to study different aspects of human disease. In all species, induction of hypercholesterolemia accelerates and exacerbates atherogenesis, but within a given experimental situation, individual animal plasma cholesterol correlates poorly with extent of atherosclerosis, indicating major importance of other factors as determinants of lesion development. Individuals have been identified in 2 primate species[26,27] which exhibit high and low plasma cholesterol responses to cholesterol-fat feeding. In rhesus monkeys, plasma cholesterol response is related to intestinal absorption of cholesterol[26], whereas in the squirrel monkey it is inversely related to ability to increase bile acid excretion[27]. The squirrel monkey has been reported[28] to respond to dietary fats and cholesterol in a manner similar to man, and to develop arterial lesions. The rhesus monkey responds to cholesterol feeding by development of hyperbetalipoproteinemia, thus producing a model of human type II disease[23,29]. This species develops arterial disease resembling human atherosclerosis in several important ways[29,30], but over a relatively long time period[23]. In another study striking differences in lesion composition have been reported in 3 other primate species fed the same atherogenic diet[31]. The stumptail macaque experienced the greatest plasma cholesterol increase, and this was associated with lipid-poor fibrous plaques. Squirrel monkeys and African green monkeys had similar, lesser cholesterol responses, but green monkeys

produced more typical (anthropoid) atheroma, while the squirrel monkey had a variable lesion response, with greater medial involvement.

Induction of hyperlipidemia and arterial lesions in dogs requires elimination of thyroid function, in addition to cholesterol feeding. Under these conditions, dogs develop extreme hyperlipidemia resembling human type IV or type V (hyperprebetalipoproteinemia, without or with chylomicronemia); arterial lesions develop relatively rapidly, and range from fatty streaks to ulcerated plaques[23].

Pigeons develop highly predictable spontaneous atherosclerosis, which can be enhanced by dietary means, and which resembles human atherosclerosis[25]. The pathogenesis of the spontaneous lesion in susceptible and resistant strains has been further investigated[32], and the advantages of this model for metabolic studies discussed. The model has been used to discover differences in lipid composition of aorta smooth muscle, in vivo and in tissue culture, between the susceptible and resistant strains[33].

The iris[34] and cornea[35] of the cholesterol-fed rabbit have been studied as models of cholesterol deposition and removal. Severe lipid accumulation and necrosis occurs in the smooth muscle cells of the iris, but not in the iris arteries. In the cornea, lipid deposition occurred chiefly in the form of droplets (liquid crystals) of cholesterol combined with phospholipid, which disappeared following cessation of cholesterol feeding. However, injury of the cornea during the cholesterol feeding period led to accelerated deposition of crystalline cholesterol, which was not removed on a normal diet.

A major problem in atherosclerosis research concerns the selection of animal models with plasma lipoprotein distribution like that of man, especially since two of the most common laboratory species, the rat and the dog, are known to differ markedly from man in this respect. A valuable contribution to this area is a study[36] of distribution and composition of serum lipoproteins in 34 species, comprising mammals, birds, reptiles and fish. Although there appears to be a fairly general uniformity of 2 broad lipoprotein classes (HDL and LDL), no single species presents a lipoprotein spectrum exactly like that of urban man. However, as noted above, dietary manipulation in animals can reproduce lipoprotein patterns resembling those of human pathological states, which may be highly relevant since it is likely that "urban man" differs from primitive man with regard to quantitative lipoprotein distribution, and probably presents a truly abnormal lipoprotein pattern.

Diabetes, Insulin - Numerous studies have indicated that diabetes mellitus and/or decreased glucose tolerance constitute an increased risk for atherosclerosis[37-39], and a high incidence of decreased glucose tolerance has been seen in patients with clinical manifestations of atherosclerosis[40,41]. Hyperglycemia *per se* has been invoked as an atherogenic factor, operating *via* the polyol pathway of glucose metabolism[42] or *via* stimulation of glycoprotein synthesis[43] (both insulin-independent pathways), in arterial tissue. Alternatively, several studies[44-47] implicate abnormal insulin responses

(hyperinsulinism or insulin resistance) rather than abnormality of glucose tolerance. Hyperinsulinemia, especially in the presence of hyperglycemia, can be a cause of hypertriglyceridemia[48] and indeed mild diabetics frequently exhibit this kind (type IV) of hyperlipidemia[47,49]. Nevertheless, there is considerable dissociation between hyperinsulinemia and hypertriglyceridemia[44,49] and it seems likely that other effects of insulin may be atherogenic. The implications of an atherogenic effect of insulin relate to both major forms of human diabetes, in that mild (usually maturity-onset) diabetes is characterized by hyperinsulinemia, while in insulin-requiring diabetes the insulin is administered by a non-physiological route, and unavoidably is less well controlled than it is by the healthy organism.

Perhaps the most striking relationship between diabetes and atherosclerosis is seen in premenopausal women, in whom diabetes completely eliminates the protection against coronary heart disease normally enjoyed by young women[38,37]. A very provocative publication[50], which unfortunately lacks important details of procedure and materials, has appeared describing possible biochemical bases for the effects of estrogen and insulin on coronary atherosclerosis. It is reported that coronary arteries of animals and man contain a high level of hormone-sensitive lipase, relative to other arteries. This is a lipid-mobilizing enzyme, previously described in adipose tissue, where it is activated by hormones that increase cyclic AMP levels (catecholamines, glucagon, growth hormone, etc.) and decreased by insulin. Evidence is presented which implicates estrogen as a stimulator and insulin as a suppressor of this enzyme in coronary arteries. In another approach, White[51] has shown that insulin increases hepatic cholesterol synthesis in the rat, and can overcome the normal feedback mechanism by which absorbed cholesterol controls its own production. It seems very likely that insulin has an atherogenic potential when not under normal physiological control.

Clofibrate-Mechanism of Action - The position of clofibrate as the major drug available for treatment of the hyperlipidemias, and as the probable prototype of a number of agents undergoing clinical evaluation has generated intense research effort aimed at understanding its mechanism(s) of action. The greatest effectiveness of this drug is in the endogenous hypertriglyceridemias (types III and IV), and generally it lowers serum triglycerides more than serum cholesterol. In rats, reduction of plasma triglycerides precedes reduction of cholesterol[52]. It seems logical, therefore, to suspect that the primary effect of clofibrate may be exerted on triglyceride metabolism. This is by no means certain, since triglycerides, cholesterol, phospholipids and protein are secreted together into the circulation as the VLDL "package"[53], and a primary effect on any of these components could affect all others in unpredictable ways.

Attempts to determine the effect of clofibrate on hepatic triglyceride (TG) synthesis and secretion have produced conflicting results. Early studies in rats[54,55], suggested that hepatic secretion of TG into plasma (as VLDL) is reduced. At the same time, it appeared that TG synthesis was increased in the liver, even though there was only limited

accumulation of TG there. The most direct studies in man[56,57] indicate that hepatic TG synthesis and secretion into the blood are not reduced by clofibrate, but that the normal source of the fatty acid components of plasma TG, free fatty acids of plasma, is partially replaced by another source[56-58]. In contrast to these results, recent re-investigations of the effects of clofibrate on hepatic TG synthesis in the rat[59,60] have produced strong evidence for an inhibitory effect, which was attributed to inhibition of sn-glycerol-3-phosphate acyltransferase, the enzyme which initiates synthesis of glycerolipids. The substrate for this enzyme is sn-glycerol-3-phosphate (α-glycerophosphate, α-GP), and the tissue concentration of this substrate is believed to be a controlling factor in TG synthesis. Westerfeld, et al[61] and Pereira, et al[62] have shown that clofibrate administration to rats increases mitochondrial α-glycerophosphate dehydrogenase, which converts α-GP to dihydroxyacetone phosphate, and Pereira, et al found decreased liver concentrations of α-GP in clofibrate-treated rats, as well as decreased hepatic TG secretion. Thus, enzymes directly involved in the maintanance and utilization of α-GP have been implicated as sites of action for clofibrate, even though the implied result, inhibition of TG synthesis, has not been firmly established either in animals or man. Part of these apparent discrepancies may be attributed to differing nutritional status in different experiments. Precursors of hepatic TG fatty acids are different in fasting and glucose-feeding[58], for example, and it has been shown that clofibrate strongly inhibits the rise in hepatic α-GP produced by ethanol administration, but had no effect on basal levels[63]. Inhibition of acetyl CoA carboxylase, the control enzyme of fatty acid synthesis, has been attributed to clofibrate[64], but this does not agree with overall effects on fatty acid synthesis[54].

Enhancement of TG removal has been more consistently attributed to clofibrate[57,65,66], and increase of lipoprotein lipase activity, appropriate to this action, has been reported[67]. The latter effect may be secondary to inhibition of adenyl cyclase[68]. Stimulation of TG synthesis in adipose tissue of the rat by clofibrate has been reported[69].

Much work has been devoted to effects of clofibrate on cholesterol synthesis and metabolism. Gould, et al[54] showed that oral administration of clofibrate to rats resulted, after an induction period, in inhibition of cholesterol synthesis between acetate and mevalonate, and concluded that a direct effect of the intact drug is not involved. White[70] has provided definitive evidence that the primary site of inhibition is at conversion of HMG CoA to mevalonate (HMG CoA reductase). A secondary site of inhibition occurs at activation of acetate. Perhaps the most definitive human data have been reported by Sodhi, et al[71] who found that incorporation of acetate, but not of mevalonate into serum and bile cholesterol was reduced following clofibrate administration; an effect on hepatic cholesterol synthesis similar to that in the rat is strongly implied. In the dog, clofibrate did not inhibit cholesterol synthesis from pyruvate in liver, intestine or kidney[72]; important changes in glucose metabolism were observed, however. Intestine has been recognized as a potentially important source of blood cholesterol that responds to control mechanisms different from those of liver[73]. Intestinal cholesterol synthesis has been

reported to be inhibited by clofibrate in man[76] and hamster[74], but not in rats[75].

The ultimate purpose of hypolipidemic therapy is to reduce or prevent deposition of lipids in artery walls, and to provide a milieu favorable to removal of existing deposits. It is nearly impossible, with present techniques, to demonstrate achievement of these objectives in man during life, but it would be reassuring to be able to show that a total body negative sterol balance had been achieved, i.e. that a therapeutic regimen was capable of removing sterol stores from the body as a whole. To this end, a group at Rockefeller University has painstakingly developed methods for quantitative estimation of cholesterol absorption, endogenous cholesterol excretion and biliary cholesterol secretion[76]. Utilizing these techniques in 24 patients, representing all categories of hyperlipidemia, on long-term treatment with clofibrate, the most consistent effect was increased excretion of neutral sterol; this was usually accompanied by decreased excretion of bile acids, with a net increase in excretion of cholesterol-derived materials. Measurement in 3 patients disclosed increased secretion of cholesterol in bile. In patients with spontaneous or induced intestinal cholesterol synthesis, clofibrate reduced the synthesis. The most important finding was decreased size of total body cholesterol pools, measured in type II patients. Analysis of plasma cholesterol decay curves and other data strongly suggested mobilization and excretion of cholesterol stored in tissues. It was concluded that clofibrate has 2 major effects in man: inhibition of cholesterol synthesis and mobilization from tissues. These studies were conducted on liquid formula diets of varied composition.

It has been previously reported[77] that nicotinic acid increases biliary secretion of cholesterol without increasing bile acid secretion. It appears that the two most effective systemically acting hypolipidemic drugs (clofibrate and nicotinic acid) share this common effect. Aside from suggesting the importance of biliary sterol secretion, these findings generate concern over possible production of lithogenic bile[78].

Clinical Efficacy Trials - Krasno and Kidera have reported[79] effects of clofibrate on morbidity and mortality from coronary heart disease in two male populations of different average age (47.5 and 37.8 years). Treatment with clofibrate resulted in significant reductions in non-fatal myocardial infarction and total events referable to coronary heart disease, without regard to previous overt expression of disease (myocardial infarction, angina pectoris). It is remarkable that no treated individual developed angina. The onset of the protective effect of clofibrate appeared to be rapid, and was maintained for the full 5 1/2 years. Although the study as a whole validated the relationship of hyperlipidemia to coronary heart disease, protection apparently extended to hyperlipidemic individuals whose blood lipids were not reduced by treatment. This, along with the rapid onset, led to a tentative conclusion that the protective effect of clofibrate is due to some action other than lipid-lowering. A similar implication developed from the Newcastle and Scottish studies (see last year's review). Unlike the Newcastle study, however, the present

study did not provide evidence for a relationship between protection and pre-existing angina. The Krasno and Kidera report, which concerns a primary prevention trial, offers considerably more convincing evidence for a beneficial effect of clofibrate in coronary heart disease than did the Newcastle and Scottish studies. Such an outcome is reasonable on the basis that less advanced disease should be more amenable to therapy. The Newcastle and Scottish studies have been extensively discussed in the medical literature[80-82].

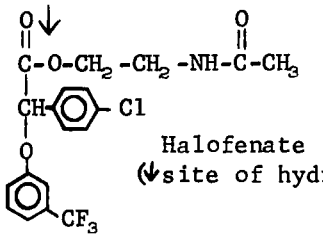
Clofibrate appeared ineffective in a small, 7-year trial for secondary prevention of cerebral vascular disease[83], in patients selected not only for evidence of CVD, but also for hypercholesterolemia. This is perhaps not surprising in view of the relatively small contribution of hypercholesterolemia to total risk in cerebral vascular disease[84].

Dextrothyroxine ($D\text{-}T_4$) has been withdrawn from the Coronary Drug Project, due to excess mortality associated with the drug[85], although the possibility remains that $D\text{-}T_4$ may be beneficial in certain patient categories.

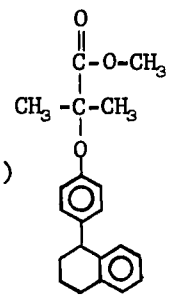
New Drugs - Although nafenopin (SU 13,437) has been withdrawn from clinical investigation, the compound continues to elicit interest. Evidence for long-term hepatotoxicity in man has been published[86]. Mechanism of its hypotriglyceridemic effect in man is reported to be reduction of plasma triglyceride production[87]. The previously reported inhibition, in vitro, of liver acetyl CoA carboxylase has now been extended to in vivo conditions[65]. Optical isomers of nafenopin have been prepared and studied for effects on lipogenic enzymes[88]; no essential difference was observed between isomers, but results are difficult to interpret in terms of in vivo lipogenesis. Hepatomegaly due to nafenopin and clofibrate have been studied in mice[89].

Halofenate continues to be characterized as a hypotriglyceridemic and uricosuric drug, with little effect on cholesterol[90,91]. Its uricosuric effect has been studied in the chimpanzee[92]. Partial hydrolysis in vivo yields "halofenate free acid", which is the uricosuric agent. Halofenate has effects similar to clofibrate on oxidation of cholesterol by rat liver mitochondria[93].

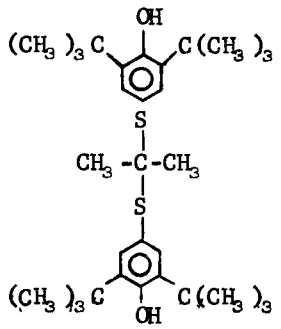
Probucol was investigated[94] in type II patients at increased dosage, and produced good (27%) cholesterol lowering. Increased bile acid excretion occurred during the cholesterol fall. Increases in excretion of water, fat and dietary cholesterol were also noted; changes in intestinal flora, fat digestion and lipid absorption are suggested. Conflicting evidence was obtained with regard to effect on cholesterol synthesis.



Halofenate
(↓site of hydrolysis)



Nafenopin

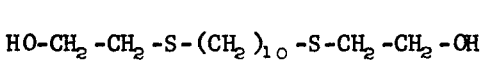


Probucol

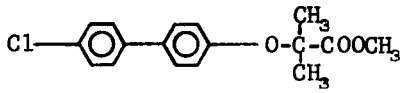
A recent study[95] suggests that the antilipolytic effect of nicotinic acid is the result of inhibition of adenyl cyclase.

A Chondroitin sulfate preparation is under continued study[96,97] as an agent potentially active directly against the atherosclerotic lesion.

Miscellaneous Drugs - Rachev, et al have described[98] a potent atherogenic effect of dietary sodium for rabbits, and an anti-atherogenic effect of sodium-depleting diuretics. A large number of antilipolytic analogs of nicotinic acid have been described[99]. A series of papers[100] has appeared on a compound, designated LL 1558, with biological effects similar to clofibrate but with a completely different structure (below). Methyl clofenapate, a clofibrate analog with very long biological half-life, produced greater reductions of low-density and very low-density lipoproteins in man than did clofibrate, but also produced long-term hepatotoxicity[101].



LL 1558



Methyl Clofenapate

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Chapter 20. Steroids and Biologically Related Compounds

Duane F. Morrow, Duane G. Gallo, Mead Johnson Research Ctr, Evansville, IN

Introduction - The flow of steroid publications slackened considerably in 1972. The rigid steroid skeleton was still used as a convenient framework for the investigation of new reactions, but many of the compounds prepared seemed to have little medicinal utility. In this review, we have attempted to select for inclusion those steroid articles which might have particular interest for the medicinal chemist.

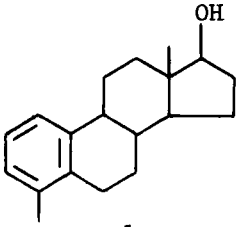
Progestins - Analogs of progesterone containing new combinations of substituents on the pregnane nucleus continued to be synthesized. Several 6-substituted 17α-acetoxyretroprogesterones were reported to be potent oral progestagens, with the 16-methylene-6-fluoro-Δ^6- and the 1β,2β-methylene-6-fluoro-Δ^6-derivatives being the most potent agents yet described.[1,2] It is interesting to note the difference between the retro and normal series in the effects of various substituents in the A and B rings on progestational activity. In the normal series the 6-substituents enhance activity in the order H<CH_3<F<Cl, with the chloro compound being approximately 6 times more potent than the unsubstituted derivative, while in the retro series the same substituents rank CH_3<H<Cl<F, with the fluoro analog being about 40 times more potent than the methyl derivative.[1,2,3] In the normal series the introduction of a Δ^1-double bond has little effect upon activity but in the retro series 1,2-dehydrogenation decreases potency.[2] Addition of a 4-chloro substituent in the normal series enhances activity but similar substitution in the retro series has little effect.[2]

Progesterone derivatives with carbon-linked substituents at C-6, such as trifluoromethyl, cyano, and formyl, were all considerably less active than those with the 6-methyl group.[4,137] The effect of 6,6-gem-difluoro substitution appears to be erratic; enhancement of activity by a factor of 2 to 4 is found in norethindrone and its esters,[5,6,7] no effect was found in the 17α-allenyl derivative[5,8] or norgestrel,[7] and a diminished activity (ca. 1/5) was found in both the progesterone and 19-norprogesterone derivatives.[9] The 17α-allenyl analog of norethindrone is 20 times more potent orally than the parent, although the corresponding 17α-allenylestradiol is considerably less potent than the 17α-ethinyl compound.[5,8] It was postulated that the greatly enhanced activity of the 17α-allenyl progestagen indicates a possible chemical reaction between the allene group and the receptor protein. Shortening or lengthening the C-17α-acyl residue of 17α-acyloxyprogesterone derivatives was shown to diminish progestational activity.[3]

A large series of acetals of 14α,17α-dihydroxyprogesterone and its derivatives was prepared, and several of these had significant progestational and antifertility activity.[10] The progestational activity of some 14α,17α-ethanoprogesterones and the synthesis of 14α,17α-ethano-Δ^{15}-progesterone, the "inverted" isomer of the progestationally active 14α,17α-ethenoprogesterone, was reported.[11,12] The synthesis of the BCD all-cis

steroid, 8α,14β-progesterone, was described, but no biological data was presented.[13] However, 8α-methylprogesterone was found to be considerably less active than the unsubstituted 8α-progesterone and less than 5% as active as progesterone itself.[14] A series of new derivatives of 16α,17α-dimethylprogesterone was prepared,[15] and variously alkylated cyclohexano- and cyclohex-3-eno[16α,17α]progesterones were found to have progestational activity.[16]

Two reports dealing with the intriguing concept of irreversibly inactivating the progesterone receptor by selective alkylation were published. In one a series of 2-chloromercuriprogesterones, which have the potential for irreversible interaction with protein-SH groups, were prepared but no biological data were reported.[17] In a second study 16α-acetoxy-, chloroacetoxy-, and bromoacetoxyprogesterone were found to possess similar activity as substrates for the 20β-hydroxysteroid dehydrogenase from Streptomyces hydrogenans, but only the 16α-bromoacetoxy compound inactivated the enzyme or alkylated several amino acid nucleophiles. Further studies with tritiated 16α-bromoacetoxyprogesterone suggested the presence of a histidine moiety in close proximity to the active site of the enzyme.[18]



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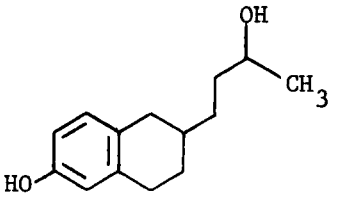

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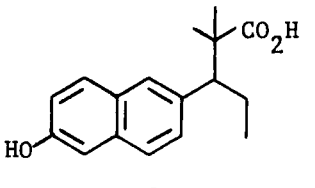

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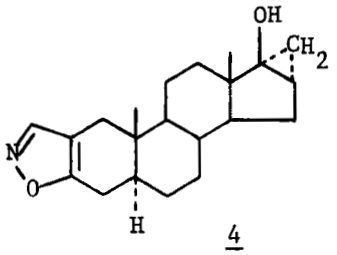
Estrogens - Investigation of the structural requirements necessary for steroidal estrogenic activity continued. The greater potency of 8α-methylestradiol, in contrast to the inactivity of the 8β-methyl isomer, indicates that the exact shape of the rigid steroid backbone separating the two bonding oxygen functions is not too important as long as no substituents are present on the β-face.[14,19] The flexibility allowable in the inter-oxygen distance was again demonstrated by the estrogenic potency (ca. 1/2 that of estradiol) possessed by the 4-hydroxy isomer 1.[20] However, D-bishomoestradiol retained only about 1% of the potency of estradiol,[21] and the C,D-bissecosteroid 2, prepared as a simpler, more estradiol-like analog of the potent estrogen Horeau acid 3, was inactive.[22] These results indicate that the nature and rigidity of the backbone separating the two bonding sites must still play an important role. The total synthesis of the optically active form of 6-thiaestrone was reported,[23] as well as the preparation of several of its variously substituted derivatives.[24]

Androgens - The oral androgenic and anabolic activities of a series of 16α,17α-cyclopropanoandrostanes were reported, with the isoxazole derivative 4 having the best anabolic/androgenic ratio.[25] The potency of 4 was less than that of the standard anabolic agents with which it was compared,

but whether the smaller cyclopropano substituent avoids the hepatotoxicity usually encountered with compounds possessing the 17α-methyl group was not discussed. In contrast with 8α-testosterone, which possesses about 40% of the androgenicity of testosterone, 8α-methyltestosterone was only very weakly androgenic but retained about 20% of the anabolic potency of testosterone.[14] The structure-activity relationships of a series of cycloalkenyl ethers of testosterone and its derivatives was reported. Several of these orally active compounds were superior to methyl testosterone in potency and/or myogenic/androgenic ratio.[26] D-Bishomotestosterone has only 3% of the androgenic potency of testosterone in chickens and is inactive in rats; however, the configuration of the hydroxyl group of both this compound and the corresponding estradiol derivative mentioned above was not rigorously established.[21] Enhanced androgenic and anabolic activity was attributed earlier to B-homo-A,19-bisnorandrostene derivatives, and a method of synthesis of these compounds was reported.[27] The preparation of 9α-cyano-19-nortestosterone was described, but no biological data was presented.[28]

A series of 17-spiroethers and spirolactones was reported to have interesting anti-androgenic activity unaccompanied by other biological activities, except for some very weak aldosterone-inhibition.[29] A non-steroidal isobutyranilide was surprisingly reported to possess anti-androgenic activity equipotent to that of cyproterone acetate with no other endocrine-related effects. It also did not interfere with libido or sexual potency in male rats.[30]



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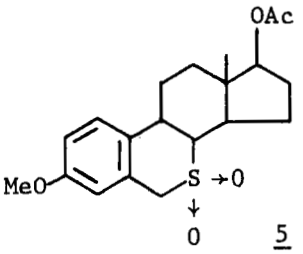

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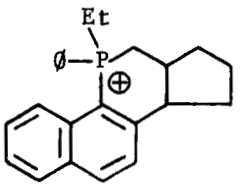

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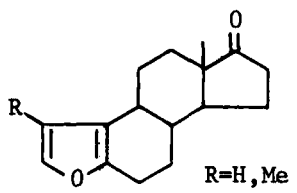
Corticoids - The first syntheses of an 18-methyl and a B-nor corticoid were reported but biological properties were not discussed.[31,32] A 21-oxazolidino corticoid had 4 times the potency of hydrocortisone in the thymus and granuloma reduction assays.[33] Some 17,21-diesters of 6α,9α-difluoroprednisolone were found to be 10 to 35 times as active as the parent triol,[34] and several other derivatives of known anti-inflammatory steroids were reported to possess advantageous biological properties.[35-37]

The isolation of a group of $\Delta^{9(11)}$-steroids, mostly 3β,6α-dihydroxy-5α-pregn-9(11)-en-20-one, from starfish raises the interesting possibility of using these as a convenient starting material for synthesis of 11-oxygenated steroids.[38-43] A new procedure for synthesizing the corticoid side chain from a 17-ketone was reported.[44]

Hetero Steroids - Much work continues to be done in this area, including

many new syntheses of ring systems described earlier. Among the many interesting new compounds reported were 11-oxaprogesterone and some of its derivatives. The parent compound was considerably weaker than progesterone in the McPhail-Clauberg assay, but was 2 1/2 times as potent as progesterone as an ovulation inhibitor in rabbits.[45] The oral estrogenic potency of 8-azaethinylestradiol was only slightly less than that of ethinylestradiol.[46] The syntheses of a 7-thiaestradiol derivative (5)[47] and the first phosphasteroid (6) were reported.[48] The 2-aza analog of oxandrolone and its 19-nor derivative, as well as 2-azaestrone methyl ether and the corresponding 17α-ethinyl derivative, were synthesized, but no mention was made of their biological activity.[49] The synthesis of the A-furano steroids 7 was described.[50] A series of 3-hetero-A-homoandrostan-17β-ols was prepared, and in contrast to the inactive A-homotestosterone, several of these possessed anabolic and androgenic activity.[51]



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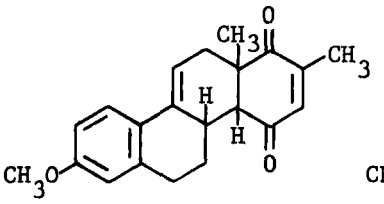

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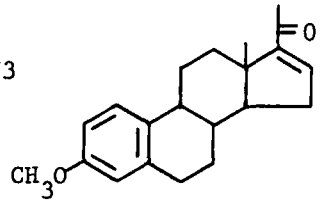

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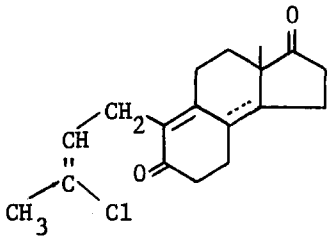
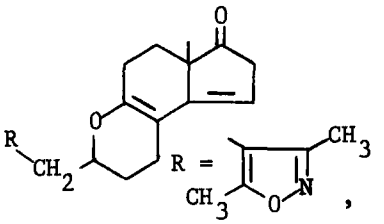
Total Synthesis - Condensation of a vinyldihydronaphthalene with 2,6-dimethylbenzoquinone afforded the key intermediate 8 in 69% yield. This was readily isomerized to the 14α-epimer and converted by standard means to 9 in 22% overall yield. This relatively simple synthesis affords 20-ketopregnanes, with or without the useful $\Delta^{9(11)}$-double bond, and should be equally applicable to the more challenging 3-keto-Δ^4-10β-methyl series, for which the authors reported encouraging initial results.[52,53] The use of 2-methyl-6-vinylpyridine as an alkylating agent which can be easily converted to a β-(3-oxo-1-cyclohexenyl)ethyl group afforded an easy route to D-homo-19-norsteroids.[54] The authors are attempting to adapt this procedure to the synthesis of the normal 5-membered-D-ring steroids. A new procedure for the introduction of the 19-methyl group into the tricyclic intermediate 10a by alkylation of the anion of the dienone was reported.[55] The relative values of a variety of precursors for the A ring in the syntheses of estr-4-ene-3,17-dione proceeding through the enol ether intermediate 11 were discussed, as well as new syntheses of the intermediate itself.[56-61] The synthesis of 19(10→9β)abeo-10α-testosterone was reported, the key step being the introduction of the 9β-methyl group by the action of lithium dimethylcopper on the enone 10b.[62] The conversion of l-abietic acid to an antipodal steroid was begun, and the synthesis of a key tricyclic intermediate was reported.[63] The cyclization of a monocyclic analog of squalene oxide to the lanosterol ring system was described.[64] We can expect this process to be adapted soon to the synthesis of a variety of novel ring-substituted sterols.

Miscellaneous Chemistry - The preparation of 17-spirolactones such as 12 has been facilitated by the development of a new synthetic procedure, in



10a ($\Delta^{8(14)}$)
b (dihydro)



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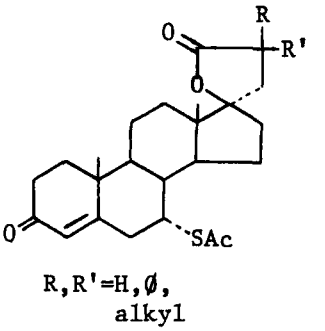

R,R'=H,Ø, alkyl

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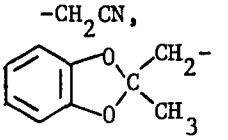
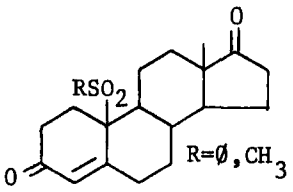


which a 17β,20-epoxide is treated with the dilithio salt of the appropriate acid.[65] It was found that the 21-hydroxyl group of the corticoid side chain is displaced by secondary amines to give 21-amino derivatives. The reaction, which occurred even more readily than enamine formation at the Δ^4-3-keto function, presumably proceeds through the tautomeric 20-hydroxy-21-aldehyde.[66] The synthesis of 14β-fluoro steroids was described,[67] as well as the use of bis(fluoroxy)difluoromethane to convert enol esters to α-fluoroketones (e.g. 9α-fluoro-11-ketones).[68] The total synthesis of 10β-sulfur substituted steroids (13)[50] and the preparation of the 17α-thiol derivative of pregnenolone[69,70] were reported, but no mention of biological activity was made. An unusual degradation of the 17β-acetyl side chain by triphenyltin hydride was described, in which the lactone 14 was formed by reduction of 3β,12α-diacetoxypregna-14,16-dien-20-one.[71] The selective reductions of steroid ketones with sulfurated borohydrides was reported.[72] A very nice unsymmetrical cleavage of the 2,3-bond of 3-keto-5β-steroids affording the cyanoaldehyde 15 was accomplished.[73]



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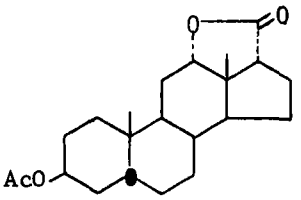

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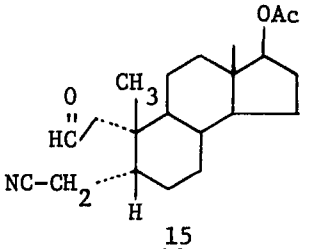


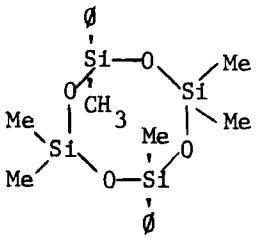
15

Oxidation of estrogens with benzoyl peroxide afforded the corresponding 2-benzoyloxy derivatives in 55-60% yield,[74] whereas the use of thallium trifluoroacetate gave in 75% yield the 10β-trifluoroacetoxy-$\Delta^{1,4}$-3-one, which was readily hydrolyzed to the 10β-ol.[75]

Remote functionalization was accomplished by a variety of new methods. Photochemical oxidation of 3β,17β-diacetoxy-5α-androstane with peracetic acid afforded a 25% conversion to a 1:1 mixture of the corresponding

5α- and 14β-hydroxylated derivatives.[76] The photochemical reaction of phenyliodosochloride (and to a lesser extent bromotrichloromethane) with saturated steroids afforded a 75% conversion to a 1:1 mixture of $\Delta^{9(11)}$- and Δ^{14}-steroids.[77] Deoxycholic acid was oxidized with air, EDTA, and ferrous ion to a 15α-hydroxy derivative.[78]

Antifertility - The cyclotetrasiloxane 16 represents the first purely non-functional organosilicon compound reported to have endocrine activity.[79] It is a weak estrogen with no antiestrogenic properties and mimics the antifertility and antigonadotropic actions of estrogens, but may possess, in addition, a direct antiandrogenic action.[79-81] Four tetrahydroisoquinolines were reported to have properties similar to those of impeded estrogens but to display postcoital antifertility effects only at estrogenic doses.[82] In contrast, a series of 4-methyldibenzothiophenes possessed properties typical of impeded estrogens and two of these were claimed to show antifertility effects not explicable by their estrogenic potency.[83] Similarly, some cyclic ethers of ethinylestradiol[84] and estriol[85] were reported to possess postcoital antifertility properties at non-estrogenic dose levels. A series of anil intermediates in the synthesis of 12-aza-steroids were reported to have an anti-implantation effect,[86] and one of a series of 4(3H)-quinazolines had weak postcoital antifertility activity in the rat.[87] Dihydroelactericin A (17) was reported to have only weak uterotropic activity but to cause prolonged inhibition of ovulation.[88] In a brief report, the oral administration of sodium acetate was claimed to have antifertility effects in mice, hamsters, guinea pigs and rabbits at doses ranging from 1 to 200 mg.[89] The postcoital antifertility activity of a series of aminoalcohols in the mouse, rat and rabbit was presented in a preliminary report.[90] Confirmation of these reports and elucidation of the modes of action of these agents may provide pertinent information concerning the chemistry and pharmacologic control of reproduction. Reviews on the effects of nonsteroidal compounds in nonhuman primates,[91] and the mode of action of nonsteroidal antiestrogens[92] were published.



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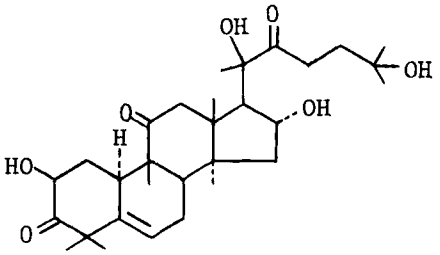
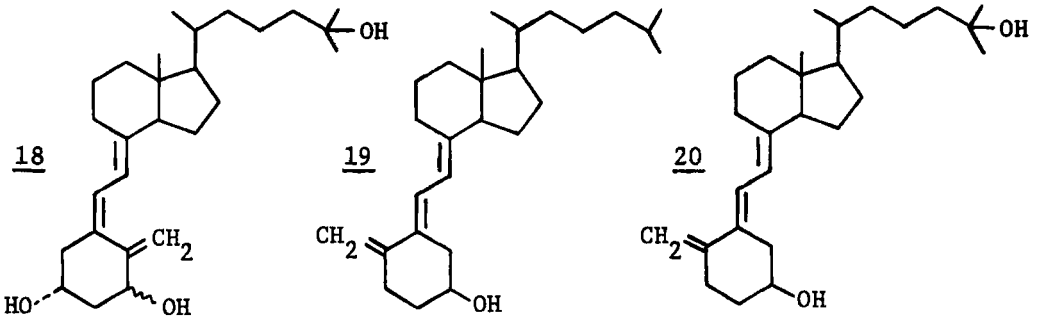


17

Vitamin D - Continued investigations have clearly demonstrated that cholecalciferol (Vitamin D_3) must be hydroxylated, first at C-25 by the liver and then at C-1 by the kidney, before it can directly function in calcium homeostasis.[93-98] The resulting 1,25-dihydroxycholecalciferol (18) can stimulate both the intestinal absorption[98] and the mobilization[94] of bone calcium. From limited structure-activity studies it appears that a

hydroxyl at C-1 of the vitamin D molecule is required for promotion of these biological effects.[94,96] Thus 5,6-trans cholecalciferol (19) can directly stimulate bone calcium mobilization. Interestingly 5,6-trans-25-hydroxycholecalciferol (20) is capable of stimulating the intestinal absorption but not the mobilization of bone calcium, a fortuitous combination of properties which makes this compound an attractive agent for the treatment of the abnormalities in calcium homeostasis which occur in chronic renal failure. The recent synthesis of 1α,25-dihydroxycholecalciferol served to confirm the configuration of the natural metabolite and should provide sufficient quantities for clinical investigations.[99] Two new allene derivatives were isolated as by-products from the irradiation of cholecalciferol in ethanol but their biological activity was not investigated.[100]



Radioimmunoassay - One of the most vexatious problems encountered in biological studies of steroids is the inability to accurately and specifically measure the vanishingly small quantities normally present in biological tissue or fluids. An exciting recent development in this field is the realization that steroids, attached as haptenes to protein molecules, elicit the formation of antibodies which are directed against the particular steroid used as the haptene. The most important determinant of the specificity of the antibodies for a given steroid appears to be the locations of the functional groups of the steroid relative to the site of its attachment to the protein antigen. As might be anticipated, the specificity of the antibody is enhanced by attaching the steroid to protein at a site as far as possible from the functional groups which distinguish the steroid.[101-103] The chemistry involved in preparing suitable antigens is described in several papers.[101-105] A particularly intriguing procedure for derivatizing Δ^4-3-ketones involves nucleophilic attack on the 6,7-dehydro derivatives by ambidentate reagents to give 7α-thioether alkanoic acids, which may then be attached to protein via e.g. the ε-amino group of lysine.[102] Steroids for which radioimmunoassay methods were published in 1972 include: Δ^5-3β-hydroxysteroids;[106] aldosterone;[107-111] corticosterone;[112] deoxycorticosterone;[113] cortisol;[114,115] 11-deoxycortisol;[116] dehydroepiandrosterone;[117] estrone, estradiol and/or estriol;[101,103,104,118-120] 15α-hydroxyestriol;[121] pregnenolone;[122] progesterone;[123-126] 17-hydroxyprogesterone;[127] and testosterone.[128-130] In an intriguing modification of the usual procedure, an antibody to estradiol was covalently

linked to a water insoluble carrier, thus facilitating the separation of bound and unbound labeled steroid.[131] A general review of radioimmunoassays was also published.[132]

Miscellaneous - Review articles on steroid photochemistry,[133] the synthesis and biological activity of aza steroids,[134] and the pharmacological activity of heterosteroids[135] have appeared. The two-volume set, "Organic Reactions in Steroid Chemistry", provides a very useful reference work.[136]

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Chapter 21. Peptide Hormones of the Hypothalamus and Pituitary

Wilfrid F. White, Abbott Laboratories, North Chicago, Ill. 60064

Due to the great increase in the number of papers on the two subjects involved, some attempt must be made to limit the size of this review. This will be done by slanting this presentation toward the chemical, as opposed to the biological, aspects of these hormones. In the case of the hypothalamic hormones this approach will avoid overlap with an extensive recent review by Schally et al.[1]. The greater emphasis on chemistry is also in accord with the fact that Peptide Synthesis is not a subject for review in this Volume. The author is indebted to S. Jane Weinstein of the Abbott Library Staff for assembling and abstracting pertinent references.

Hypothalamic Peptides

The large review[2] anticipated last year has apparently not yet reached general circulation. However, one very comprehensive review[1] and several general reviews of the physiological and clinical aspects of the hypothalamic releasing hormones have appeared[3].

Thyroid Stimulating Hormone (TSH) Releasing Hormone, TRH - TRH continues to attract the attention of many clinical investigators. The most exciting new development is the finding that TRH causes a prompt improvement in depression. The first announcement, made in abstract form by Prange and Wilson[4], was rapidly followed by a more complete report by the same authors[5], an additional paper by another group of investigators[6], and several brief editorial comments. The mode of action of TRH in depression is still not understood, although the authors of the two major papers have presented some speculation. Plotnikoff et al.[8] have attempted to illuminate the mode of action of TRH by means of an animal model. Surprisingly, these authors found that hypophysectomy had no effect on the reaction of TRH in the "dopa plus pargyline" test[9] in the mouse. Since the "dopa plus pargyline" test was devised to screen drugs for antidepressant activity, the authors speculate that the antidepressant effect of TRH in humans may not be dependent on thyroid hormone action.

Several excellent reviews of the better known clinical effects of TRH have appeared[10]. The last of these, a review by J. F. Wilber, scheduled to appear in mid-1973, is a very comprehensive general review on TRH. Shorter reviews have appeared on the physiopathology[11] pituitary function reserve[12] and cancer[13] aspects of TRH in man.

Clinical testing of the prolactin-releasing effect of TRH briefly mentioned in last year's Review, has continued. Tyson et al.[14] have shown that the post-TRH elevation of prolactin, especially in the later phases of lactation, augments the production of breast milk. In the same paper the authors attempt to use the pattern of responsiveness of prolactin to exogenous TRH to build a comprehensive theory of the role of

prolactin in the human. Other attempts at an overall view of the role of prolactin and of TRH as a releaser of prolactin have been made by Friesen[15] and by Bowers et al.[16].

After the intensive synthetic activity of the previous year, in which more than 50 analogs of TRH were produced, there is very little to report this year. One paper[17] describes a single analog, Pro-His-Pro-NH_2. Unfortunately, no bioassay data are included. A second new analog, reported by Sievertsson et al.[18] is the [Phe^2] compound which, having 10% of the potency of TRH, is described as "the most potent analog where one of the natural amino acids of TRH is replaced by another common natural amino acid." Overall, however, the [(τMe)-His^2] analog previously described[19] is still the most potent TRH analog yet encountered. Several attempts have been made to approach the theoretical basis for structure-function relationships. Grant et al.[20] have studied the effect of various substituents on the pKa of the imidazole proton which in TRH has a value substantially below that for free imidazole (6.25 vs. 7.08). Data for the analogs suggest a hydrogen-bonding interaction between the N^{π}-imidazole nitrogen and the first peptide bond amide nitrogen. Modification of the [Glu moiety or of the C-terminal amide nitrogen moiety had only minor effects on this interaction, while substitution on the N^{τ}-nitrogen atom of the imidazole ring stabilizes the hydrogen bonding as shown by a lower pKa value, thus yielding a biologically hyperactive analog. In a study of the [Phe^2] analog[18], it was speculated that "both the π-electrons and the basicity of histidine may be functional for ultimate release of thyrotropin; release may consist of both complexing and an ionic mechanism involving a negatively charged group of the receptor site."

The multiple biological activities of TRH brings up the interesting question of the interrelationships of these activities to chemical structure. As yet no important information has appeared on this point. Bassiri and Utiger[21] have developed a highly specific radioimmunoassay for TRH, sensitive to 0.1 nanogram. This is still far too insensitive to detect levels of TRH in the peripheral circulation, but the test has permitted the same authors[22] to study the inactivation of exogenous TRH by serum in vitro.

Synthesis of radio-labeled TRH by ^{14}C-histidine[23] and by ^{3}H-histidine[24] has been reported. By the use of labeled TRH, Morin et al.[25] have studied the kinetics of distribution of the hormone in the male rat and Warembourg[26] has done a histoautoradiographic study of the hypothalamus after hormone injection. Warembourg found that tracer penetrated the hypothalamus but could not demonstrate specific labeling of any particular area of the gland. Two TRH binding site studies have been reported. Labrie et al.[27] have isolated plasma membranes from bovine anterior pituitary glands showing specific binding capacity for TRH of the order of 600 femtomoles per mg protein and have been used to develop an assay for the binding of ^{3}H-TRH. Wilber and Seidel[28] have isolated a 10,800 x g rat pituitary particulate fraction with high binding affinity for TRH. The fraction from propylthiouracil-treated animals was more active than from normals. The authors conclude that TRH-mediated TSH secretion is

dependent on TRH binding to a specific thyrotroph plasma membrane receptor site. Mitwick and Reichlin[29] have published a more extensive report in confirmation of their earlier finding that TRH is synthesized enzymatically. They have designated this enzymatic activity as "TRH Synthetase."

Gonadotropin Releasing Hormone (Gn-RH) - The decapeptide pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, which was isolated from porcine and ovine glands, identified and synthesized prior to the period of this review, is also called LH-RH/FSH-RH. In the opinion of this reviewer, however, another alternative designation "LRH" or "LRF" is no longer proper since the decapeptide has been shown to release both LH and FSH. Also, there is preliminary evidence for the existence of a compound specifically releasing FSH and there seems to be no theoretical reason why a compound specifically releasing LH will not be found in tissue extracts or synthesized accidentally at some time in the future. These still hypothetical substances would then require the designation FSH-RH and LH-RH, respectively. The close association of the two releasing activities in the decapeptide is attested to by the fact that the author has not seen any significant dissociation of these activities among over 100 analogs (cf. Table 1 for a partial list) already examined.

With regard to the subject of a separate FSH releasing hormone, evidence has been presented[30] for the existence of such a substance both in direct extracts of porcine hypothalami and in bio-synthetic homogenates of porcine hypothalamic tissue. The primary test method used in this study was the release of LH and FSH from the pituitaries of twenty-day old female rats *in vitro*. The samples tested were relatively crude fractions made by gel filtration of partially purified extracts. Since the samples which showed primarily FSH-releasing activity were closely contiguous to those showing the usual dual release pattern for Gn-RH, the experiments do not seem to rule out the conclusion that a secondary factor is working to modify the action of Gn-RH either by augmenting FSH release or by suppressing LH release. As in the case with Gn-RH, it will require isolation and characterization of the new factor before a judgment can be made regarding its physiological role.

After more than a year of wide availability of synthetic Gn-RH decapeptide, a great deal of animal testing has been reported. A current publication from the author's laboratory[31] describes experiments in the ovariectomized estrogen-progesterone treated rat where LH is the primary gonadotropin released, tests in the anestrous ewe where both LH and FSH are released and induction of ovulation in the rabbit. Others have described LH release in the ewe and ram[32], cattle[33], pig[34] and dog[35]. Induction of ovulation by means of synthetic Gn-RH has been achieved in the golden hamster[36], the rat[37], rabbit[38] and ewe[39]. A more detailed review of the effects of synthetic Gn-RH in animals is presented in the review by Schally *et al.*[1].

Soon after the synthesis of Gn-RH had been achieved, it was shown[40] that both natural and synthetic pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-

NH_2 released LH from ovariectomized estrogen-progesterone treated rats with identical dose regression lines. As experience with the synthetic decapeptide accumulated, it was found that LH and FSH were often released together, but there have also been clear indication that FSH release is sometimes absent. In an attempt to explain at least some of these discrepancies, Arimura et al.[41] have shown that a slow infusion of Gn-RH in intact mature male rats results in significant concomitant dose-related, serum increases of both LH and FSH, whereas rapid administration gives only LH release. In summary, much additional evidence will be needed to determine the exact role of Gn-RH in gonadotropin release and to determine whether or not additional as yet unknown factors are present in the hypothalamus.

The possible role of Gn-RH in human reproduction has been reviewed.[42] Papers on the clinical use of synthetic Gn-RH in men[43], women[44], and children[45] have appeared. Other publications[46] summarize the effects in both sexes. To date most clinical papers have been probing the sensitivity of normal and abnormal patients to the effects of Gn-RH. However, three papers[42,43,47] claim the induction of ovulation in women by the use of Gn-RH.

Bio-synthetic conversion of ^{14}C-glutamic acid to the gonadotropin releasing hormone has been reported by Johansson et al.[48]. A Pauly-reactive spot corresponding to the Gn-RH decapeptide exhibited radioactivity after incubation and purification of the hypothalamic tissue homogenate. The presence of newly synthesized LH-releasing activity was confirmed by bioassay.

A double antibody radioimmunoassay has been developed to measure Gn-RH[49]. The assay has been shown to be highly specific by testing against a long list of synthetic analogs representing possible degradative products of the decapeptide. Direct measurement of serum levels of Gn-RH in normal rams showed 71 picograms per ml as compared to 128 pg/ml in the long-term castrate ewe. The half-disappearance time of Gn-RH in sheep after intracarotid administration was of the order of 7 minutes.

Synthetic work on Gn-RH has been rapidly accelerating. At least 12 reports have appeared describing analogs of Gn-RH which are equivalent in chain length to the natural molecule and which involve simple deletions or substitutions by other naturally occurring amino acids[50-61]. These analogs, together with the relevant bioassay data, are shown in Table 1. From the work summarized in Table 1, it seems fair to draw the conclusion that the biological activity of Gn-RH is sensitive to changes in all of the amino acid positions which have been studied thus far and that substitutions by similar amino acids are least likely to abolish activity. To date, however, manipulations involving the naturally occurring amino acids have not resulted in agonists more active than the parent decapeptide nor in antagonists with marked activity. A more successful approach to the design of useful agonists has been that employed by Fujino et al.[62] who have concentrated on subtle changes at the C-terminus. In a series of compounds made by replacing the glycine amide structure by substituted

amides on the proline residue in position 9, several members of the series were found to be markedly more active than Gn-RH both *in vitro* and in "ovulatory index". The most active member of the series (des-Gly-NH_2^{10}, Pro-ethylamide^9) which proved to be of the order of five times as active as Gn-RH, is the subject of a more detailed report[63]. In a third paper, Fujino *et al.*[64] summarize the chemical properties and biological activities of 15 members of this series of analogs. The potencies of these compounds vary from more than 500% to less than 1% that of the natural hormone structure. This work points out in unprecedented detail the rich possibilities for analog work even in a restricted portion of a peptide molecule.

Growth Hormone (Somototropin) Releasing Factor, GRF or SRH and Release Inhibiting Factor, SRIF - Last year's Review mentioned a decapeptide which was isolated from porcine hypothalamic extracts, using test systems based on stimulation of the release of bioassayable growth hormone. However, in a variety of *in vivo* test systems the decapeptide, in synthetic form, did not cause growth or the release of radioimmunoassayable growth hormone, resulting in the conclusion by Schally *et al.*[1] that "it is unlikely that the decapeptide is the physiological GH-RH." More recently several groups[65] have presented evidence for a factor, chromatographically distinct from the Schally decapeptide, which causes release of radioimmunoassayable growth hormone. To date, however, nothing further has been reported on the identification of this substance.

Very recently, Brageau *et al.*[66] have announced the isolation from ovine hypothalamic extract of a tetradecapeptide H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH which "inhibits the secretion *in vitro* of immunoreactive rat or human growth hormone and is similarly active *in vivo* in rats." The peptide has been synthesized and it has been shown that the synthetic compound duplicates the activity of the pure natural product. The new peptide has not yet been proven to be the only natural factor for the negative control of GH release, but it is reasonably active in the test systems used and deserves additional study in an attempt to evaluate its physiological role.

Prolactin Releasing Hormone, PRH, and Prolactin Release Inhibitory PIF - As was reported in last year's Review, no meaningful chemistry on the nature of these factors has been published. Recently, however, Valverde *et al.*[67] have presented evidence confirming the existence of a prolactin releasing substance in porcine and in rat hypothalamic extracts distinct from TRH and other recognized hormones of the hypothalamus. In view of the increasing examples of "species differences" it should be said that Valverde *et al.* used the rat as the test animal in their experiments and, therefore, their results may apply only to the rat. Meanwhile TRH, as detailed in an earlier section of this Review, appears to be a very efficient releaser of prolactin in man. To date there does not appear to be any evidence of a specific prolactin releasing substance in man.

Melanocyte Stimulating Hormone (Release) Inhibiting Factor, MIF, and

Releasing Hormone, MRH - Since there are several peptides which have been reported to be inhibitory toward MSH release, there is still the possibility of confusion in using the term MIF. However, since the tripeptide Pro-Leu-Gly-NH_2 was the first inhibitor to be isolated from hypothalamic extracts, it will be designated MIF in this Review. Other inhibitors of MSH release will be referred to by their structural designation. No MSH-releasing peptides, have yet been isolated and identified from hypothalamic extracts. Therefore, all such compounds will also be referred to by their structural designation. The question as to which of the MSH release inhibiting peptides is the true natural hormone is still unsettled. The problem is complicated by lack of agreement as to choice of assay and, perhaps, also by species differences. Using the *in vivo* rat assay, Celis *et al.*[68] have studied the inhibition of MSH release by several analogs of MIF. Of those tested only the Lys^2 and Arg^2 analogs were active, but these two were markedly less active than MIF (Pro-Leu-Gly-NH_2) itself. These investigators also found that the MSH-release inhibiting pentapeptide fragment of oxytocin, H-Cys-Tyr-Ile-Gln-Asn-OH, is inhibited by MIF and several of its analogs. Tocinoic acid, the ring component of oxytocin, H-Cys-Tyr-Ile-Gln-Asn-Cys-OH, was inactive both in the release of MSH and in inhibition of MSH release in the rat.

In seeking possible clinical uses for MIF, Kastin *et al.*[69] have studied the interactions between the pineal, hypothalamic and pituitary glands of the rat with respect to melatonin, MIF and MSH. The authors conclude that their animal studies may have eventual clinical application since MIF potentiates certain behavioral effects of DOPA in mice[70] and is effective in reversing signs resembling those of Parkinsonism or depression in monkeys receiving deserpine and in mice pre-treated with oxotremorine[71]. Two reviews in the general subject of brain-endocrine interaction have recently appeared[72].

Pituitary Hormones

Despite the large amount of brilliant fundamental work which is being reported, this subject will be treated very briefly due to space limitations. This course of action seems justified because most of this work has not yet had a direct impact on human medicine.

Growth Hormone (GH or STH) - The chemistry of growth hormone and the relationship of GH to prolactin are the subjects of a review by Niall *et al.*[73]. The amino acid sequence of human GH, the details of which were settled during the previous year, has been compared by Li *et al.*[74] with the sequences of the bovine and ovine hormones. As expected, the ovine and bovine molecules are much closer to each other than either is to the human molecule. The authors conclude that "these differences may provide some clues as to differences in the biological profiles between human and animal growth hormones." Yamasaki *et al.*[75] have isolated a 37-amino acid fragment of bovine growth hormone which retains some of the biological activities of the present molecule.

Prolactin, PRL - It is now established that human PRL and human GH are discrete and separable. Friesen[15] has reviewed the implications of this knowledge toward clinical practice.

Glycoprotein Hormones: Thyroid Stimulating Hormone, TSH, Luteinizing Hormone, LH, Follicle Stimulating Hormone, FSH - Two extensive review papers bearing upon the subject of LH structure were presented at the Laurentian Hormone Conference of 1972. At that time there was very general agreement on the amino acid sequence of ovine LH between Ward,[76] speaking for the University of Texas group and Papkoff,[77] speaking for the University of California group. Subsequently, the latter group has published[78] separately the complete sequences of the ovine α- and β-subunits and appear to have rationalized any small differences which had existed previously between them and the Texas group. In a previous paper the University of California group had published the sequence of the α-subunit from human ICSH (LH), which has 7 fewer amino acid residues than its ovine counterpart with a homology of about 70%. These results, together with previous indications of close relationships between LH-α and TSH-α and between LH-β and TSH-β explain many of the early troubles with immunological overlaps between these hormones. Exact knowledge of the sequences of the various subunits should be helpful in a study of their immunochemical interrelationships. Reichert et al.[79] have detailed the application of a radiological receptor assay to the study of LH and its subunits.

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Table 1: Analogs of Gn-RH

Compound	Ref	Potency (Gn-RH=100)
Position 1: [Glu		
des-[Glu^1]	54	.002[d]
0 =(Ser^1	57	5-25[a],6[b],7[c]
0 =(Thr^1	57	<<5[b],0.2-2[c]
Pro^1	57	<0.1[a],<0.1[b]
Position 2: His		
des-His^2	53	<0.001[e]
Gly^2	53	non-parallel[e]
(π-Me)-His^2	53	3[e]
(τ-Me)-His^2	53	6[e]
Phe^2	57	4-7[a],2[b],0.2-2[c]
Lys^2	57	<0.1[a],[b],[c]
Arg^2	57	<0.1[a],[b]
(τ-Me)-His^2	57	1[a],1-2[b],0.4[c]
Position 3: Trp		
Phe^3	58	50[e]
Position 4: Ser		
Gly^4	61	1.5[e]
Ala^4	55	5[d]
Ala^4	57	3-6[a],16[b],2[c]
Thr^4	57	4[a],17[b],2[c]
Gln^4	57	8[a],6[b],2-20[c]
Position 5: Tyr		
Phe^5	58	50[e]
Cl-Tyr^5	57	8[a],5[b],2-20[c]
di-Cl-Tyr^5	57	<1[a],[b],[c]
Phe^5	60	64[d]
Position 6: Gly		
None		
Position 7: Leu		
Gly^7	57	3[a],5[b]
Ala^7	57	5-6[a],3-5[b],2[c]
Val^7	57	16[a],20-35[b],20[c]
Ile^7	57	45[a],33[b],35-55[c]
Nle^7	57	30[a],22[b],35[c]
Position 8: Arg		
His^8	52	~0.5[d]
Nva^8	52	~0.5[d]
des$_8$Arg^8	52	<0.5[d]
Lys^8	59	~2[d]
Gln^8	56	2-5[d]
Leu^8	56	0.5-0.8[d]
Pro^8,Arg^9	56	0.02[d]
Lys^8	57	11-28[a],25[b],10[c]
Orn^8	57	6-12[a],5[b],10[c]
Position 9: Pro		
none		
Position 10: Gly-NH$_2$		
Gly-OH^{10}	50	~0.1[d]
Ala^{10}	57	~10[a],2-20[c]
des-Gly^{10} Pro-NH$_2$^{9}	53	11[e]

Key to assay methods used:

[a] *in vitro*, LH-release

[b] *in vitro*, FSH-release

[c] ovulation inducing activity

[d] in vivo, OEP-treated rats

[e] other methods

Chapter 22. Non-steroidal Antiinflammatory Agents

Marvin E. Rosenthale, Wyeth Laboratories Inc., Radnor, Pa.

Introduction - The search for non-toxic and broad spectrum antiinflammatory agents continued unabated in 1972 despite little progress in delineating the etiology of the chronic inflammatory diseases. Some progress was made on the mechanism of action of the non-steroidal antiinflammatory agents (NAA). Major topics covered in review articles included the clinical evaluation of NAA and their use in the inflammatory diseases;[1-4] clinical guidelines for immunosuppressive therapy;[5-7] and the pharmacology and chemistry of antiinflammatory agents.[8,9]

Pathogenesis - Exogenous stimuli (microbial or viral), the immune responses of the host, and the genetic components of the host response were intensively studied as the keys to etiology of the rheumatic diseases.[10,11] Viral antigens were described in nephritis, arteritis and arthritis. The possibility was entertained that non-bacterial arthritis, which has been associated with rubella, leukemia and viral hepatitis, may be a consequence of immune complexes between viral antigen and host antibody.[12] The remission of rheumatoid arthritis by thoracic lymph duct drainage underlined the importance of lymph cells in sustaining clinical articular inflammation, thus confirming similar experiments in rat adjuvant arthritis.[13] The importance of lysosomal mechanisms in joint inflammation was reviewed,[14] and intravascular degranulation of neutrophils was shown to be present in urate-induced synovitis in dogs.[15] Antigen-induced synovitis in rabbits was studied to provide support for the concept that antigen persistence (as in mycoplasma arthritis) is responsible for prolonged immunologic responses associated with cellular infiltration in joints.[16] Cyclic AMP-mediated events were shown to be critical in the activation and regulation of immuno-competent cells,[17] suggesting possible new approaches to regulation of immunologic phenomena. The isolation and identification of the as yet unidentified local antigen(s) which participate in the immunoinflammatory response characteristic of the chronic inflammatory diseases, appears presently to be the most critical obstacle to real progress in this area.

Mechanism of Action - The biochemical basis for the actions of the NAA remained unclear. Although considerable effort was expended in investigating the effects of antiinflammatory drugs on mucopolysaccharide metabolism, no clear correlation with drug effects was seen.[18] The prostaglandins (PG) E_1 and $F_{1\alpha}$ were shown to be capable of increasing collagen synthesis *in vitro* in a manner similar to that observed in inflammation.[19] Additional evidence was obtained indicating that some PG's may be mediators of inflammation, including the incapacitating effect of PGE_1, E_2 or $F_{2\alpha}$ when injected into the dog knee joint[20] and the release of PG's in ocular inflammation in the rabbit.[21,22] These pro-inflammatory effects of the PG's were extensively reviewed.[22-24] Previously reported paradoxical therapeutic effects of PGE_1 and E_2 were studied in detail in

the rat with adjuvant-induced arthritis (AIA) and were found to be related to the stress induced by large doses of these agents.[25]

Inhibition of PG synthesis by drugs as a mode of antiinflammatory action continued to command attention. Aspirin was shown to prevent ocular inflammation in the rabbit,[26] while several NAA were shown to inhibit cholera toxin-induced fluid accumulation in the gastrointestinal tract of rats,[27] both effects being attributed to inhibition of PG synthesis. Inhibition of PG synthesis by certain corticosteroids, which was shown for the first time using human skin,[28] may be relevant to the mode of action of topical steroids. Inhibition of PG synthesis as a mode of action of NAA is supported by the correlation which was noted between such inhibition and the antiinflammatory activities of these drugs in several experimental models.[29-32] However, an analog of fenclozic acid, [2-(4-methoxyphenyl)-thiazol-4-ylacetic acid] was described which was found to be capable of inhibiting PGE_2 synthesis in-vitro yet to be devoid of antiinflammatory activity, and its existence may cast some doubt on this theory.[33] This lack of correlation was examined by Vane,[34] who correctly pointed out the hazards of comparing oral activity in animals with drug effects on enzymes in a cell-free system. Evidence accumulated thus far strongly suggests inhibition of PG synthesis as a probable mode of action of at least some of the effects of NAA.

The prevention of migration of leucocytes as provoked by dextran-induced pleurisy,[35] the suppression of their phagocytic function as induced by urate crystals in the knee joint,[36] and the inhibition of the release of lysosomal enzymes from migrating leucocytes were additionally considered as possible mechanisms of action for both steroidal and NAA.[37,38]

Paradoxically, the stabilization of lysosomes by a protein in the serum was demonstrated in the AIA rat, and in vitro labilization of lysosomes by several antiinflammatory drugs was proposed as a factor in diminishing tissue injury in this experimental pathology.[39,40] A recent symposium emphasized the complex array of factors and events presently implicated in the inflammatory process.[41]

Test Systems - If curative rather than palliative agents are to be found, increased efforts to devise test systems capable of examining drug effects on various chronic tissue-destroying mechanisms are needed, rather than the presently used quick, non-specific assays.[42] Some progress was made in this area with the use of the local graft vs. host; activated lymphocyte passive transfer; and Arthus reactions as assays to study drug effects.[43-46] A symposium on models for studying rheumatoid arthritis suggests that greater emphasis be placed on chronic rather than acute experimental models.[47]

The AIA rat continued to be a popular model for evaluation and differentiation of various classes of both systemic,[48-50] and topical[51] antiinflammatory drugs. Collagen metabolism in the AIA rat was found to

be initially intensified, then retarded in the acute and chronic stages.[52] Chronic polyarthritis, as induced in the rabbit with Escherichia coli,[53] in the rat with Mycoplasma arthritidis,[54] and in the pig with Erysipelothrix rhusiopathiae,[55] were proposed as additional models for drug evaluation. Experimental allergic encephalomyelitis (EAE), induced in the rabbit[56] or the rat,[57,58] was used to evaluate the effect of a variety of drugs on the immune response.

The well defined similarity of spontaneous immunologically mediated glomerulonephritis of NZB/W mice to human systemic lupus erythematosus makes this animal model unique and useful for drug evaluation. The relative efficacy of azathioprine, cyclophosphamide and methylprednisolone and the synergistic effects of a combination of all three drugs in this disorder suggested an experimental basis for combination trials in human lupus erythematosus nephritis.[59,60]

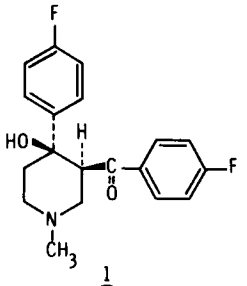
Miscellaneous interesting test systems that were developed included the use of sodium urate-induced edema in the rat to investigate the antimitotic and antiinflammatory actions of colchicine and its derivatives;[61] the modification by NAA of the three phases of the inflammatory response to cotton pellet implantation in the rat;[62] and two human tests, one based on skin response to the tuberculin or histamine reaction[63] and the other on the ability of antiinflammatory drugs to delay the onset of erythema after ultraviolet irradiation.[64] The rat continued to be useful for describing the gastrointestinal irritating properties of the NAA,[65] but a more predictive model is urgently needed.

Compounds Under Investigation

Immunosuppressants - Antiinflammatory agents possessing greater therapeutic ratios, longer durations of action and, hopefully, curative rather than palliative powers continued to be sought. Agents acting through control of immunopathologic mechanisms continue to offer great promise, but non-specific toxicity, and a generally long onset of clinical activity, makes evaluation difficult and costly and severely limits the number of clinically tested agents despite the large number of interesting drugs available. The antiphlogistic activity of a number of agents was studied in some experimental models.[66] Several excellent reviews on immunosuppressants and their clinical applications in inflammation were published.[5-7,41,67-69] The principles expounded in these reviews remain to be tested in the clinic, utilizing controlled double blind long-term studies with careful monitoring of immunologic phenomena.

A basic agent R-760 (flazalone) (1) exhibited a unique antiinflammatory and mild immunosuppressive spectrum of activity in animals and in some limited trials in man. This agent was equipotent with phenylbutazone in experimental models; had no analgesic and weak antipyretic activity; but was as effective as azathioprine in goldfish scale and rabbit-ear skin homotransplantation experiments. It also inhibited nucleic acid synthesis of lymphocytes in-vitro.[70,71] Cycloleucine was

described as a unique type of immunopharmacologic agent. It was effective in the adjuvant and allergic encephalomyelitis rat models but was not cytotoxic in-vitro and had no effect on polymorphonuclear leukocyte migration.[39,44,50] The toxicity of this agent could be reduced by L-valine which was shown to act by increasing cycloleucine excretion.[72] Oxisuran (2) was characterized as an immunosuppressant with the important difference of being capable of suppressing allograft immunity without concomitant effect on humoral antibody formation. The 3- and 4-positional isomers did not show the selective immunological properties of the 2-isomer. Oxisuran is reportedly effective on cutaneous delayed hypersensitivity in guinea pigs and rats sensitized to ovalbumin, tuberculin or DNCB. It is not cytotoxic or antiproliferative in-vivo or in-vitro nor is it antiinflammatory.[73] If clinical trials substantiate these claims these agents could be the forerunner of the selective immunosuppressants which have been discussed for years but not as yet realized.



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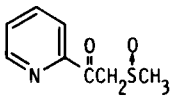

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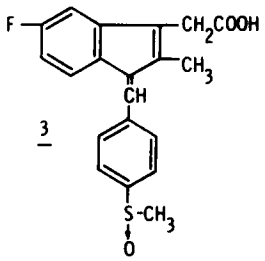


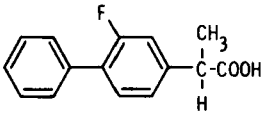
3

Arylalkanoic Acids and Related Compounds - The combination of a p-methylsulfinyl substituent as a solubilizing agent and a fluorine group to increase the potency of the indene isostere of indomethacin resulted in a new series of compounds (3) having a pharmacologic profile similar to that of indomethacin but with fewer gastrointestinal side effects in animals.[74] A double blind crossover trial showed indomethacin (100 mg) to be superior to the salicylate aloxiprin (3.6 g) and both to be superior to placebo in rheumatoid arthritis and ankylosing spondylitis.[75] The human plasma concentrations and protein binding capacity of indomethacin were studied using a sensitive fluorescence assay,[76] and the human metabolic pathways of the drug and its metabolites were extensively studied using an isotope dilution method.[77] It was demonstrated in normal human volunteers, using a specific spectrofluorometric method which detects only unchanged indomethacin, that the concurrent administration of aspirin did not affect serum indomethacin levels. Thus, the interaction (antagonism) of these two agents reported in animals and man was not mediated through changes in total serum concentrations of indomethacin, as previously suggested by non-specific isotopic labeling methods.[78] The series of indan-1-carboxylic acids discussed last year continued to be explored; the (-) isomer BL-236579 was claimed to inhibit platelet aggregation, to be effective in the potency range of indomethacin, and to have a better separation of therapeutic from ulcerogenic properties in

several experimental models.[80,81] Despite reported activity and low toxicity in animal studies, clamidoxic acid[79] was shown ineffective in a study in 24 patients with rheumatoid arthritis.[82]

New *in vivo* and *in vitro* studies have shown fenoprofen to be a more effective inhibitor of collagen-induced platelet aggregation than aspirin or phenylbutazone.[83] Metabolic studies of this compound in man indicated that phenylbutazone, but not indomethacin or aspirin, can influence its ability to bind to plasma albumin.[84] In a collaborative open, non-comparative study in 201 patients, ibuprofen at 600 to 1200 mg daily was found devoid of side effects while effectively relieving morning pain and stiffness in patients with a variety of rheumatic conditions.[85] The more potent analog flurbiprofen (4) proved effective and well tolerated in a double-blind crossover trial on a short term basis in patients with rheumatoid arthritis.[86] Absorption, distribution, metabolism and excretion of naproxen was studied in animals and humans; the human plasma half-life ranged from 10 to 17 hr.[87]

SU-21524 (5) was studied in 16 patients with rheumatoid arthritis in a randomized double-blind crossover trial. A daily dose of 400 mg was as effective as 100 mg indomethacin.[88] In a double-blind crossover study involving 46 patients with rheumatoid arthritis and 42 patients with osteoarthrosis of the hip, Orudis (RP-19583) was well tolerated and had comparable therapeutic efficacy with indomethacin when given at equal doses (100 mg daily dose).[89]



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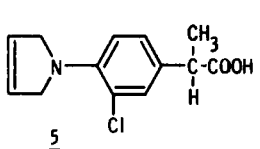

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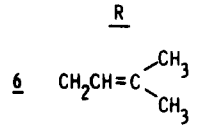
Salicylates - Benorylate, an esterified aspirin preparation, caused significantly less gastric blood loss than soluble aspirin in both volunteers and arthritic patients.[90,91] A controlled double blind study demonstrated that benorylate, 6-8 g daily, was as effective as 150 mg indomethacin in patients with rheumatoid arthritis.[92]



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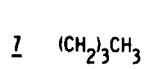

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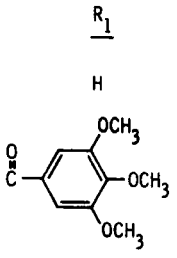
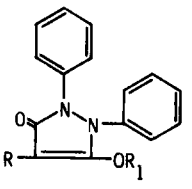
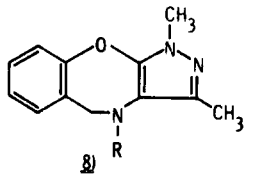




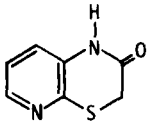


8)

Pyrazoles - Prenazone (DA-2370)(6)[93] and LH-150 (7) were similar to phenylbutazone in experimental antiinflammatory activity, although the blood levels of the latter were lower than phenylbutazone.[94] A series

exemplified by 8 was found to have similar antiinflammatory activity in rats but was less toxic than phenylbutazone.[95]

Benzothiazines and analogs - In rats, a new benzothiazine analog, Abbott 29590 (9), had an antiinflammatory potency between that of phenylbutazone and aspirin, was low in toxicity and produced little gastric irritation.[96] Sudoxicam (10) demonstrated activity in several animal models of inflammation in the potency range of indomethacin.[97] In man it had an extended half life of 24-26 hr, which resembled that of phenylbutazone and was much longer than that of indomethacin, thus offering the advantage of more sustained therapeutic blood levels.[98] A 1,4-benzothiazine, SQ-11579 (11), demonstrated marked antiinflammatory activity and lack of ulcerogenic activity in the rat.[99]



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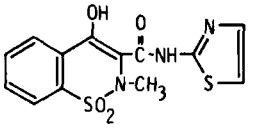

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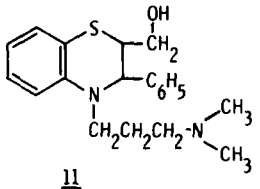
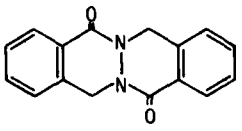


11

Miscellaneous - L5418 (12) demonstrated sufficient antiinflammatory activity in the rat to warrant clinical trial.[100] W-2354 (13) at doses of 0.5 to 1.8 g/day caused a hypouricemic response and some central nervous system effects within 2 to 3 days in patients with gout.[101]



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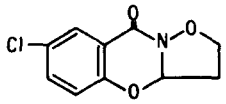

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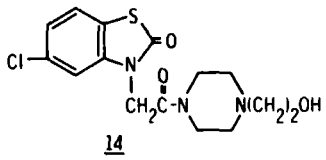
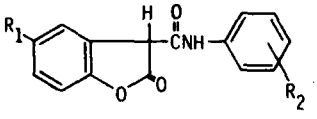


14

The basic agent, tiaramide (14), was found comparable to the basic drug benzydamine in its antiinflammatory spectrum in rodents.[102] A series of carboxanilides (15) exhibited activity equivalent to or better than aspirin in the rat carrageenin-induced foot edema assay.[103] Bimetopyrol (16) was the most potent of a series of neutral diaryl pyrroles when tested both prophylactically and therapeutically in the AIA rat.[104]



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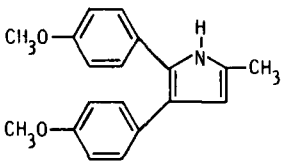

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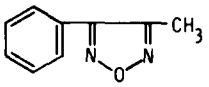
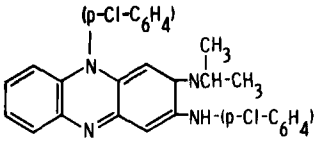


17

Phenylmethyloxadiazole (PMO) (17) was as effective as phenylbutazone in preventing the respiratory inflammation induced in guinea pigs by acrolein aerosol.[105] The anti-leprotic agent clofazimine (18) inhibited rat adjuvant arthritis but had no immunosuppressive activity.[106]



18

A multicenter trial group report of a twelve-month double-blind study showed oral D (-) penicillamine to be an effective treatment for active rhematoid arthritis. Adverse effects such as rash, nausea and vomiting, thrombocytopenia and proteinurea were reversable upon drug withdrawal. The use of patients with advanced disease may have contributed to the high incidence of side effects. The potential hazard to the kidney is the most serious drawback to this agent.[107] In a double-blind two-center trial of 60 patients with rheumatoid arthritis L-histadine at 4.5 g/day for 30 weeks showed no significant difference from controls in measurements as grip strength, walking time, numbers of swollen joints etc. There was some indication that patients with long duration of disease may have shown some improvement.[108]

Natural Products and Related Substances - A peptide bound to circulating proteins in human serum was found to have antiinflammatory properties, as indicated by its ability to reduce carrageenin-induced rat paw edema.[109] An antiinflammatory substance synthesized in the liver and found in inflammatory exudates was purified and separated from its irritant component.[110] The biological properties of carrageenin were reviewed, and its antiinflammatory and immunologic properties emphasised.[111] The experimental and clinical pharmacology of dimethyl sulfoxide was reviewed.[112] An essential oil extracted from Zanthoxylum budrunga compared favorably with standard antiinflammatory agents in rats; clinical activity was also claimed but not documented.[113] The interaction of sulfated polysaccharides with polymorphonuclear leukocytes was proposed as a mode of their antiinflammatory actions.[114] Sodium fluoride proved effective in the AIA rat, potentiating the effects of triamcinolone in this test at non-toxic doses.[115] In a series of in vitro and in vivo test systems, ascorbic acid was shown to have weak in vivo antiinflammatory activity.[116] Many of the "antiinflammatory" activities proposed for the substances mentioned in this section undoubtedly are due to their moderate toxic or stress effects and/or to the selection of the appropriate (favorable) experimental model by the investigators.

Gold - Evidence has continued to accumulate supporting the beneficial effects of chrysotherapy in rheumatoid arthritis,[117] thus stimulating clinical studies correlating drug disposition and metabolism with therapeutic effects. A positive correlation between antirheumatic activity and low fecal gold excretion was found,[118] but serum gold levels did not relate to either toxicity or clinical response in two separate studies.[119,120] Sequestration of gold around synovial tissue rather than in

synovial fluid or plasma was proposed as a possible explanation for this lack of correlation.[121] The anti-injury as well as immunologic mechanisms of action of gold preparations were studied in pathophoric lymph node cell transfer experiments using rat EAE as the model.[45] An orally administered gold compound SKF-36914 (triethylphosphino-gold chloride) was as effective as parenteral gold in suppressing inflammatory lesions of adjuvant arthritic rats.[122]

Comment - In general, the types of agents pursued in the laboratory in 1972 were related to established drugs and did not represent significant advances. Reports on the clinical investigation of agents with unusual spectra of activity are needed before any breakthrough can occur. The large number of agents now in the clinic (reputedly more than thirty) including some of the newer more selective immunoregulants may open new avenues to further experimental efforts in this area.

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Chapter 23. Agents Affecting Cyclic AMP Levels

Don N. Harris, Nick S. Semenuk and Sidney M. Hess
Squibb Institute For Medical Research, Princeton, N.J.

A comprehensive review of the 1535 reports on the action of cyclic AMP that have been published during 1972 is obviously beyond the scope of this chapter. We have chosen, therefore, to deal with publications concerned with those aspects of adenylate cyclase that seem most directly related to a disease process, and with cyclic nucleotide phosphodiesterase (PDE), the enzyme primarily responsible for the inactivation of cyclic AMP. We have also included, where appropriate, significant publications that describe mechanisms of physiologic processes involving the metabolism of cyclic AMP, which we hope will be of help to the investigator in the search for new agents that act against specific diseases.

Adenylate Cyclase - The extensive study by Weinryb et al. of a large number of drugs representing 49 therapeutic classes was designed to learn if cyclic AMP-mediated reactions were the basis for the common mechanisms by which therapeutic agents act. The spectrum of tests included, in addition to action against PDE of the rat brain and cat heart, lipolysis in isolated fat cells of the rat, steroidogenesis in isolated rat adrenal cells, and effects on adenylate cyclase of guinea pig lung.

The data indicated that cyclic AMP-mediated reactions were not a common modality by which these classes of drugs acted. Activation and inhibition of adenylate cyclase were observed, but they occurred among the classes in no discernible pattern.[1,2]

A well-executed series of studies in Greengard's laboratory has demonstrated the presence, in homogenates of caudate nucleus of rat brains, of a dopamine-stimulated adenylate cyclase. In the presence of dopamine, the accumulation of cyclic AMP was blocked by haloperidol or chlorpromazine; apomorphine, a dopamine-like agent, was also shown to be active in stimulating adenylate cyclase. The suggestion was made that increases in adenylate cyclase activity in the caudate nucleus may have beneficial results for patients with extrapyramidal diseases, such as Parkinson's disease.[3,4]

An important finding related to the stimulation of adenylate cyclase in the glial and neuronal cells of the brain was that the former may be stimulated by catecholamines and the latter by prostaglandins (PG), especially by PGE_1.[5] The glial adenylate cyclase was responsive to β stimulation and to dopamine.[6] Catecholamines injected into cerebral ventricles of the brains of rats in vivo increased the levels of cyclic AMP, whereas dopamine was barely active in this regard.[7] The stimulation of adenylate cyclase in the rat brain by norepinephrine was enhanced by pretreating the animals with 6-hydroxydopamine, an agent known to deplete norepinephrine levels in the brain.[8]

Slices of specific areas of the guinea pig brain were examined to learn the characteristics of the receptor sites of adenylate cyclase. Cyclase of cerebral cortex, hippocampus, and amygdala responded to stimulation by α-adrenergic agents and histamine.[9]

Mounting evidence pointed to the accumulation of cyclic AMP, as a result of the stimulation of adenylate cyclase in the walls of the small intestine, as the mechanism by which the enterotoxin of Vibrio cholerae caused a voluminous fluid transport into the lumen.[10,11] Cholera toxin, which was in contact with the luminal side of the epithelial cell, seemed to affect adenylate cyclase after the characteristic lag in onset of action[12] at the opposite (basal and lateral) side of the cell,[13] although the activity of the kinase was found to be in the brush (luminal side) border of the cell.[14] The activity of adenylate cyclase isolated from the jejunum of patients with cholera was shown to be increased twofold during the disease state compared with that found during recovery. It was also demonstrated that a further stimulation of the enzyme could be elicited by incubation of the biopsy specimens with PGE_1 or NaF.

An interesting study was reported by Jacoby and Marshall,[15] who demonstrated that anti-inflammatory compounds inhibited cholera toxin-mediated diarrhea in rats, which was measured by weighing the small intestine at the end of the study. Dexamethasone and prednisolone, anti-inflammatory steroids, were effective in relatively high doses. Indomethacin was the most active non-steroid tested; aspirin, indomethacin, and phenylbutazone were active when given orally or parenterally. The mechanism of action of these agents vs. cholera toxin was not determined, but it may be noted that, with the exception of the steroids, they all inhibit prostaglandin synthetase.[16] Aspirin also blocked the secretion produced by cholera toxin in isolated intestinal loops of cats.[17] Data describing the action of these anti-inflammatory drugs in humans with cholera have not yet been reported.[18]

Epinephrine, given to asthmatic patients, failed to induce the increase in concentration of urinary cyclic AMP that had been found in healthy subjects, although glucagon elicited the usual increase in both groups.[19,20] Glucocorticosteroids, such as hydrocortisone, may act to alleviate the symptoms of asthma by increasing the activity of adenylate cyclase or by restoring the responsiveness to catecholamines of the cyclase in leukocytes.[21] In this regard, α-adrenergic blockade was also found to be helpful,[22] perhaps by an enhancement of the β stimulation.

An important study reported by Kaliner, Orange and Austen[23] showed that stimulation of adenylate cyclase from human lung by β-adrenergic agents resulted in a decrease in the immunologic release of histamine and the slow-reacting substance of anaphylaxis (SRS-A). In contrast, their release was increased by the accumulation of cyclic GMP that resulted from the stimulation of guanylate cyclase by α-adrenergic agents, such as phenylephrine, or cholinergic agents, such as acetylcholine or carbamylcholine. Human leukocytes in vitro responded with a decrease in the

antigenically induced release of histamine (immediate hypersensitivity response) and a reduction in lysis of mouse mastocytoma (delayed hypersensitivity response) by sensitized lymphocytes when agents such as catecholamines, PG, cholera toxin, PDE inhibitors, or cyclic AMP were incorporated into the incubations.[24,25]

The cytolytic activity of lymphocytes of animals has been shown to be decreased after stimulation of adenylate cyclase by such agents as histamine, isoproterenol, PGE_1 and PGE_2.[26] In contrast, the cytolytic activity was enhanced by cholinergic agents, such as acetylcholine and carbamylcholine, which also increased the levels of cyclic GMP.[27] It has been suggested that the ability of interferon and polyadenylic polyuridylic acid to increase the number of antibody-forming spleen cells in mice is related to the stimulation of adenylate cyclase by these agents;[28] increased antibody formation in mice by catecholamine stimulation of adenylate cyclase has also been reported.[29]

Adenylate cyclase of platelets is an unusual enzyme because it is inhibited by epinephrine.[30,31] One other tissue, islets of Langerhans from the rat, also contains an epinephrine-inhibited cyclase.[32,33] The enzyme in platelets was stimulated by PGE_1, adenosine, (2-chloroadenosine inhibited[34]), caffeine, imipramine, and dibutyryl cyclic AMP.[35] Thrombin was found to inhibit platelet adenylate cyclase activity[36,37] even in the presence of the stimulant phytohemagglutinin from Lens culinaris.[38] Norepinephrine[39] and PGE_2[40] were reported to inhibit the stimulant effect of PGE_1; norepinephrine may have activated a degradative mechanism of cyclic AMP in platelets of humans and rabbits. The ability of PGE_2 to inhibit the action of adenylate cyclase and to enhance aggregation of platelets should be taken as a caution by those who might wish to use this agent as an abortifacient in any patient with a history of phlebitis or of cardiovascular difficulty.[41]

The findings (1) that the rate of proliferation of tumor cells in culture was reduced when the levels of cyclic AMP were increased in the incubation medium;[42-48] (2) that epidermal cells possess a complete metabolic system for the synthesis and breakdown of cyclic AMP;[49] (3) that a cyclic AMP-dependent protein kinase is present in homogenates of the skin of mice;[50] and (4) that a β-adrenergic activated adenylate cyclase is present in skin *in vivo*,[51] have prompted a number of groups to examine the cyclic AMP levels in psoriatic skin, since psoriasis is characterized by a hyperproliferation of the basal cells of the epidermis. Compared with adenylate cyclase from normal skin or from the unaffected skin of psoriatic patients, adenylate cyclase from psoriatic skin was less responsive *in vitro* to stimulation by epinephrine and was unresponsive to NaF.[52] In a similar comparison, Voorhees *et al.*[53] found a significant decrease in concentration of cyclic AMP in the involved skin of psoriatic patients. These workers also showed that the adenylate cyclase of human skin responded to the stimulation of β-adrenergic agents, such as isoproterenol, and that β-adrenergic blockade prevented the stimulation.[54] Dibutyryl cyclic AMP, added to cultures of mouse skin, decreased the rate

of mitoses in those flasks that contained a high concentration, 5 x 10^{-4}M, of the cyclic AMP analog.[55]

Several patients with psoriasis were treated for 4 mo. with a combination of levodopa and an inhibitor of peripheral dopa-decarboxylase, Ro4-4602, N'-(D,L-seryl)-N^2-(2,3,4-trihydroxybenzyl)hydrazine. The results of this limited study seemed to warrant further testing to establish whether a dopamine-sensitive adenylate cyclase does, in fact, exist in psoriatic skin and whether the treatment was truly beneficial.[56]

Studies of tumor cells *in vitro* have revealed a decreased response of adenylate cyclase to stimulation by PGE_1[57] and to ACTH in transformed cells, as compared with normal cells that exhibit controlled growth.[58] Ehrlich ascites cells were stimulated by NaF, β-adrenergic agents, and PGE_1, but not by $PGF_{1\alpha}$ or $PGF_{1\beta}$.[59] Leukocytes from patients with chronic myelogenous leukemia or chronic lymphatic leukemia exhibited greater responses to the stimulation of adenylate cyclase by PGE_1 than did normal cells.[60]

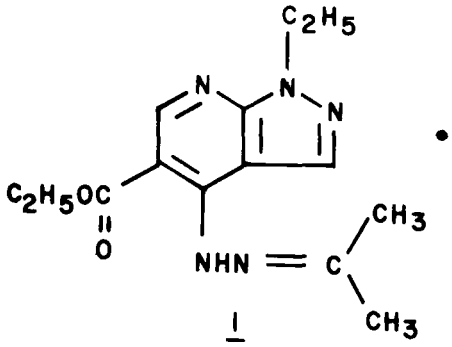
Beitch and Eakins,[61] working with rabbits, found that intracameral injections of PGE_1, E_2, $F_{2\alpha}$, A_1, or $F_{1\alpha}$ increased intraocular pressure. The potencies of the agents were: $E_1 = E_2 > F_{2\alpha} > A_1 > F_{1\alpha}$. Adrenergic agents, administered topically to the eyes of rabbits, reduced intraocular pressure and caused increases in the concentration of cyclic AMP in the aqueous humor of the eyes. The order of potency, both for reducing pressure and for increasing the concentration of cyclic AMP in the eye, was: epinephrine>norepinephrine>isoproterenol. Intracameral injections of cyclic AMP, stimated to yield a final concentration of 4 x 10^{-4}M in the anterior chamber, produced a decrease in intraocular pressure.[62]

A body of evidence collected in the past year seemed to show that cyclic AMP is involved in the release of insulin from the islet cells of the pancreas by a mechanism that includes the metabolism of glucose and Ca^{++}.[32,63,64] Islet adenylate cyclase and insulin release were stimulated by glucagon and tolbutamide.[65] The exocrine portion of the pancreas contains adenylate cyclase that was stimulated by secretin and pancreozymin; it was unresponsive to epinephrine (the endocrine enzyme was inhibited by epinephrine[66]), acetylcholine, glucagon and gastrin.[33]

Cyclic AMP Phosphodiesterase - Since PDE was discovered by Sutherland and Rall,[67] many characterization studies of this enzyme have been conducted. One major development has been the discovery that at least two forms of the enzyme exist with low and high Km values.[68] During the past year numerous laboratories have confirmed this with PDE from a variety of tissues.[69-83] Russell *et al.*[83] found, after chromatographic separation of rat brain PDE, that one form of the enzyme exhibited kinetics suggesting the existence of two enzymes or of one enzyme exhibiting negative cooperativity. By use of a computer model, they decided that negative cooperativity offered the better theoretical explanation for the kinetic

behavior of the enzyme. Several other laboratories[69,70,84,85] have extended these studies by subjecting soluble supernatant fractions of several tissues to polyacrylamide-gel electrophoresis. Campbell and Oliver[84] and Uzunov and Weiss[85] found that the electrophoretic patterns of PDE obtained from brains of rats differed from those for PDE from other tissues. Six distinct peaks of PDE activity and a discrete fraction containing a potent activator of PDE were discovered by Uzunov and Weiss.[85] Using starch-gel electrophoresis, Pichard et al.[86] also found different electrophoretic patterns for PDE from the brain and blood platelets of humans. These studies suggested that different molecular forms of PDE may exist in different cell types.

Numerous studies showed that unsubstituted cyclic nucleotides other than cyclic AMP acted as inhibitors and/or substrates of PDE isolated from many sources.[69-71,74,75,79,81,83,84,87-89] In addition, new compounds with diverse structures were investigated for their ability to serve as substrates or inhibitors of PDE. A series of new derivatives of cyclic AMP with a substituent at the C-8 position was investigated by Harris et al.[89] as inhibitors of PDE from cat heart and rat brain. The decreasing order of potency of inhibitors of the enzymes from both sources was 8-bromo cyclic AMP, followed by derivatives with a sulfur, oxygen or nitrogen atom at C-8. Chasin et al.[76] discovered a potent new compound, a pyrazolopyridine (1) that was 60 times more potent an inhibitor of rat brain PDE than theophylline.



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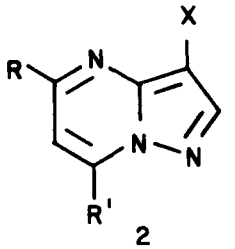

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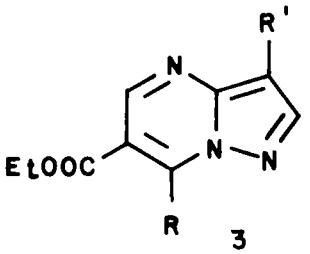

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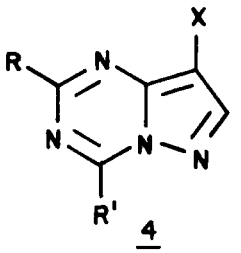

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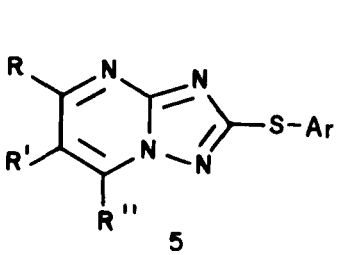

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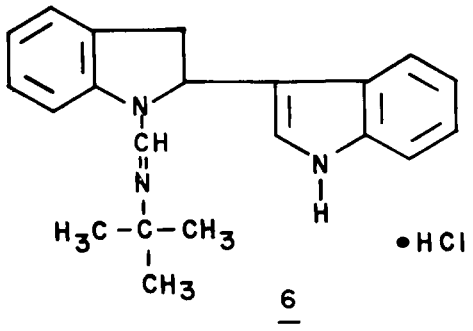
Investigators of the ICN Corporation synthesized a series of pyrazolopyrimidines,[90,91] (2,3,) pyrazolotriazines,[92] (4) and triazolopyrimidines,[93] (5) and showed them to be inhibitors of PDE from rabbit lung and

kidney and beef heart. This same group synthesized 1-β-D-arabinofuranosylpyrimidine 3',5'-cyclic phosphates that showed antitumor and antiviral activities and were slowly hydrolyzed by PDE of rabbit kidney.[94] Lake et al.[95] synthesized derivatives of 4'-thio-cyclic AMP that were hydrolyzed by PDE from bovine heart muscle. Chlorothiazide and 1-[(tert-butylimino)-methyl]-2-(3-indolyl)indoline (6) preferentially inhibited PDE of kidney from rats and guinea pigs compared to PDE from other tissues.[96] Concurrent with its diuretic effects, 6 raised intracellular levels of cyclic AMP in rat kidney.



6

Weinryb et al.[1] after examining 49 classes of therapeutic agents, found that steroidal hormones, antiparasitic drugs and several drugs active in the CNS substantially inhibited PDE activity from cat heart and rat brain. A significant correlation between anti-anxiety activity in rats and inhibition of PDE activity from rat brain was recently reported by Beer et al.[97] and Horovitz et al..[98] Pichard et al.[86] made the interesting discovery that PDE from human platelets was strongly inhibited by dipyridamole, an antagonizer of platelet aggregation, but only slightly by psychotropic drugs. The reverse was true for PDE from human brain. Investigators from two laboratories found that methyl xanthines, 3-isobutyl-1-methylxanthine, glibenclamide, tolbutamide, xylitol and leucine, all compounds that are associated with the release of insulin, were also inhibitors of PDE from pancreatic islets of mice[79] and guinea pigs.[80] On the other hand, PDE from bovine thyroid was only weakly inhibited by chlorpropamide and by tolbutamide.[74] Adenosine and some of its derivatives inhibited PDE prepared from rat adrenals[87] and from rat lipocytes and fat cell ghosts.[99] It was also of interest that PDE activity from beef heart was inhibited by catecholamines and related compounds.[100] Compounds of this sort may therefore act by stimulating adenylate cyclase and inhibiting PDE. Although the physiological significance is not clear, it was shown that nicotinamide, 3-acetylpyridine and N,N-diethylnicotinamide markedly inhibited PDE activity from rat livers.[101]

As stated earlier, Uzunov and Weiss[85] used acrylamide-gel electrophoresis to isolate a potent activator of PDE. Wang et al. [102] reported that an activator of PDE also exists in bovine heart. The synthesis of PDE was increased in fibroblasts treated with PGE_1,[103] in fat cells from adrenalectomized rats [104] and in SV 40-transformed 3T3 cells[77] treated with dibutyryl cyclic AMP and theophylline. In the latter case, synthesis of PDE was blocked by cycloheximide and actinomycin D. Imidazole stimulated the activity of PDE from pancreatic islet cells of mice[79] and guinea pigs,[80] from human placenta[105] and from rat brain.[106,107] Arginine also stimulated PDE from pancreatic islet cells of mice[79] while histamine

increased the activity of PDE from rat brain.[107] Klotz and Stock[88] presented evidence to show that low concentrations of cyclic GMP and cyclic IMP stimulated the hydrolysis of cyclic AMP in vitro, whereas higher concentrations were inhibitory. Finally, insulin stimulated the activity of PDE from a subfraction of rat liver plasma membrane,[108] and cholecystokinin and related peptides stimulated the activity of PDE from a number of rabbit tissues.[82]

There was a great increase in the number of papers that commented on the involvement of cyclic AMP and diseases in 1972 over previous years. In this chapter the most significant of these studies were discussed, with the expectation that they may provide leads for the development of new agents that may affect disease states by alteration of cyclic AMP-mediated processes.

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Section V - Topics in Biology

Editor: William J. Wechter, The Upjohn Company
Kalamazoo, Michigan

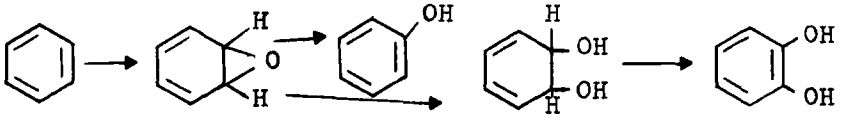
Chapter 24. Drug Metabolism

Patrick J. Murphy and Robert E. McMahon
Lilly Research Laboratories, Indianapolis, Indiana

Introduction - Drug disposition research, a field of modest scope only 15 years ago, has been growing at an enormous rate, with publications in the field now appearing at a rate in excess of 500 papers per year. The single most important responsible factor has been the development of a powerful technology involving such techniques as thin-layer chromatography (TLC), gas-liquid chromatography (GLC), and interfaced GLC and mass spectrometry (GCMS). With the recent development of methods for the combined use of heavy isotopes and GCMS even greater sensitivities and selectivities are becoming possible (1,2,3,4,5). The sophisticated technology now available, will have its' maximum value when it is brought to bear directly on investigations designed to elucidate mechanisms of drug actions and toxicities and as a tool to help develop more rational use of drugs in therapy.

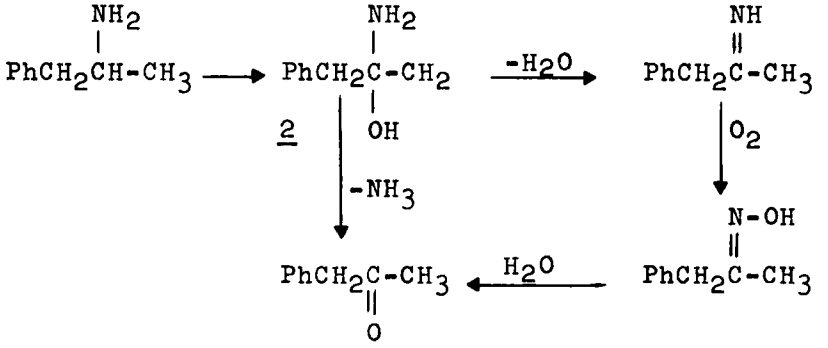
In this short review of the field we will discuss: 1. new information on enzymatic mechanisms, 2. the question of active metabolites, and 3. some selected new studies on the fate of specific drugs.

Oxidation of benzenoid systems - The initial discovery that arene oxides play an important role in hydroxylation of aromatic compounds (6) has been expanded from the naphthalene series to include various alkyl benzene derivatives (7,8) and polycyclic hydrocarbons (9,10). Although the alkyl benzene arene oxides were not stable enough to be isolated from enzyme incubations the breakdown of chemically prepared arene oxides strongly supports their intermediate role in the formation of phenols and mercapturic acids. The production of cyclohexadiene diols from benzenoid drugs such as dilantin (11), phenobarbital (12), and diphenoxylate (13) is consistent with the intermediary formation of arene oxides (Figure 1).

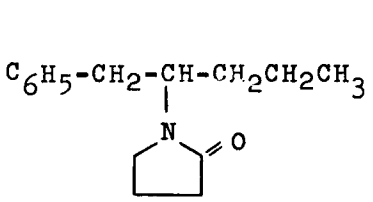


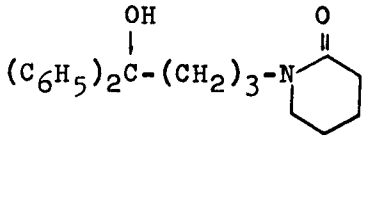
Recent work has supported the early proposals that arene oxides are key intermediates in the metabolism of polycyclic hydrocarbons (14,15). For example, dibenz (a,h) anthracene was converted to an epoxide in the presence of liver microsomes supplemented with NADPH (9). Although the exact position of the epoxide was not known in this case it has recently been reported that pyrene and benzopyrene are metabolically converted to "K-region" epoxides (10). Much further work will be needed to clearly define the role of chemically reactive arene oxides in the carcinogenic process.

Amine metabolism - Phenylacetone oxime 1 has been identified as a metabolite of amphetamine *in vitro* and *in vivo* (16). Isotope studies using $^{18}O_2$ suggest that this oxime along with carbinolamine 2 are intermediates in the deamination of amphetamine (17). The fact that imines can be converted to oximes in the presence of microsomes, NADPH and O_2 (18) suggests the following reaction course:

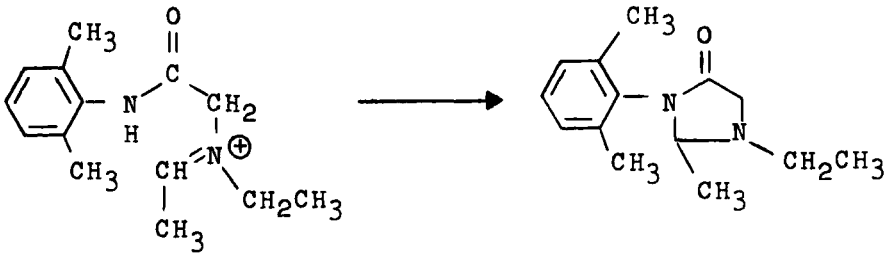


Carbinolamines have also been proposed as intermediates in the oxidation of prolintane to its lactam metabolite 3 (19) and diphenidol to its corresponding lactam 4 (20).





The elimination of water from a carbinolamine to form the iminium ion 5 has been proposed as an intermediate in the metabolism of lidocaine to the cyclic metabolite 6 (21).



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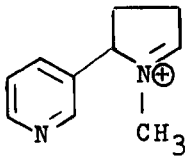
The formation of nicotine $\Delta^{1'5'}$ iminium ion 7 during the oxidation of nicotine has recently been described (22).



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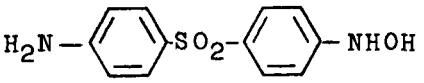


8

N-oxides have been identified as metabolites of a variety of tertiary amines including nicotine (23,24), cotinine (25), methadone (26) and orphenadrine (27). The fact that N-oxides can also be reduced back to the parent amines as in the case of imipramine (28) makes quantitation of this pathway difficult.

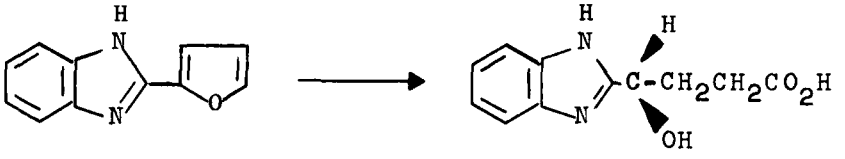
The formation of the hydroxyl amine of dapsone 8 has been proposed as the cause of methemoglobin formation seen with this compound (29,30).

Microsomal enzymes - The specificity of liver mixed-function oxidases resides mainly in the cytochrome P-450 fraction. In the reconstituted system having three necessary components, phospholipid, cytochrome P-450 reductase and cytochrome P-450, substitution of the P-450 fraction from phenobarbital induced animals for the P-450 fraction from benzpyrene induced animals results in an increase in activities normally induced with phenobarbital and a decrease in those normally induced by benzpyrene. Substitution of either of the other two fractions does not alter specificity (31,32).

The general concept that the fetal liver has a very low level of the P-450 oxygenase system has been brought into question by the finding that, based on total liver weight, the levels of P-450 in fetal liver are comparable to those of adult liver (33,34). The levels of P-450 in the fetal liver microsomes are low due to differences in the sedimentation characteristics of the enzyme system.

The finding that microsomes could catalyze the oxidation of ethanol to acetaldehyde in the presence of NADPH has stimulated a number of publications on the importance of this system in the overall metabolism of ethanol. The crux of the problem lies in whether microsomal ethanol oxidation is a P-450 related mixed function oxidase or simply a peroxidative reaction involving catalase and an H_2O_2 generating system. Rats treated chronically with alcohol show an increase in liver microsomal protein, P-450 related reactions, and the microsomal ethanol oxidizing system (35). However the rate at which P-450 levels return to normal is significantly different from the normalization of ethanol oxidizing activity (36). A careful examination of the rates of H_2O_2 formation in microsomes (37) as well as the effects of catalase inhibitors (38) indicates that H_2O_2 is produced at a rate sufficient to account for all of the increased ethanol oxidation and that catalase is required for the reaction. The mechanism involved in the induction of P-450 and related enzymes by ethanol, however, remains to be solved.

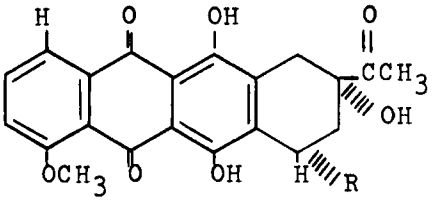
New enzymatic reactions - The enzymatic conversion of an aziridine to an alpha amino alcohol has been reported (39). Phosphines have been found to be readily metabolized to phosphine oxides (40). Metabolic cleavage of the furan ring of 2-(furyl)benzimidazole 9 gave (S)(-)4-(2-benzimidazoyl)-4-hydroxy butyric acid 10 as a urinary metabolite in five species (41):

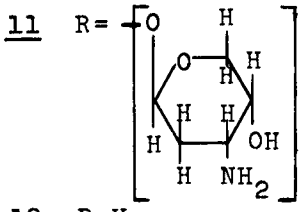


9 10

Active metabolites - The metabolism of Δ^8-tetrahydrocannabinol has recently been reviewed (42). The further metabolism of an active metabolite 11-hydroxy Δ^9 THC to 8,11 dihydroxy Δ^9 THC has been examined in man and related to the pharmacological effects of Δ^9 THC (43). Two additional metabolites of Δ^9 THC have been reported in which the C-11 methyl group is oxidized to an acid and the alkyl side chain is hydroxylated (44).

Daunomycin 11 is metabolized via a reductive glycosidic cleavage (45,46). One of the metabolites daunomycinone 12 has been suggested as a possible causative agent of cardiotoxicity seen with the parent drug (47).

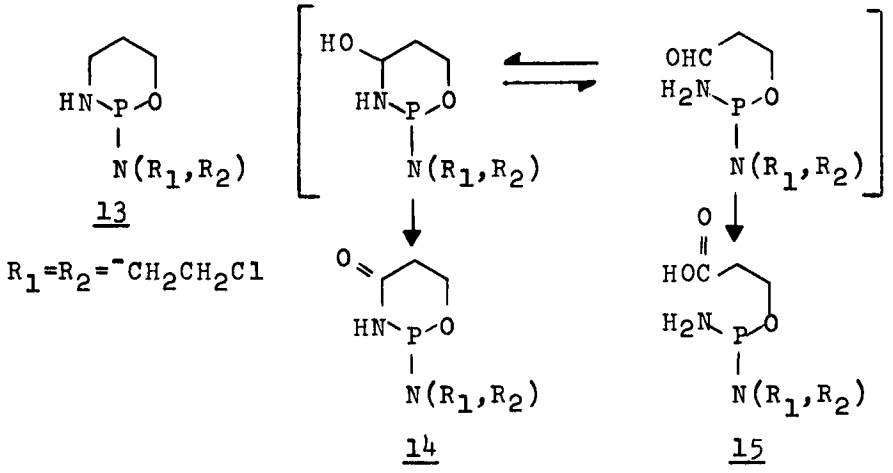




11 R=

12 R=H

Cyclophosphamide 13 is metabolized in man to 4-keto-cyclophosphamide 14 and the corresponding open chain amino acid 15 (48,49,50). In vitro liver microsomes in the presence of NADPH convert cyclophosphamide to alkylating metabolites. The production of 14 requires microsomes and 100,000 x g supernatant in a two step process similar to the production of cotinine from nicotine (51,52). In vitro metabolism of 13 also leads to the production of acrolein, another potential alkylating agent (53).



13

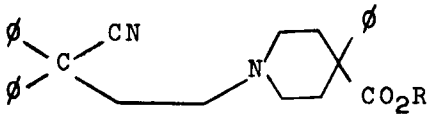
$R_1=R_2=-CH_2CH_2Cl$

14

15

Dibromodulcitol is rapidly converted to potential alkylating epoxides in vivo (54).

Diphenoxylate 16 is hydrolyzed in vivo in animals and man to produce difenoxine 17 which is more active than the parent drug (55,56,57). A minor pathway of metabolism is aromatic hydroxylation to produce phenols, a catechol and a dihydroxy cyclohexadiene derivative.

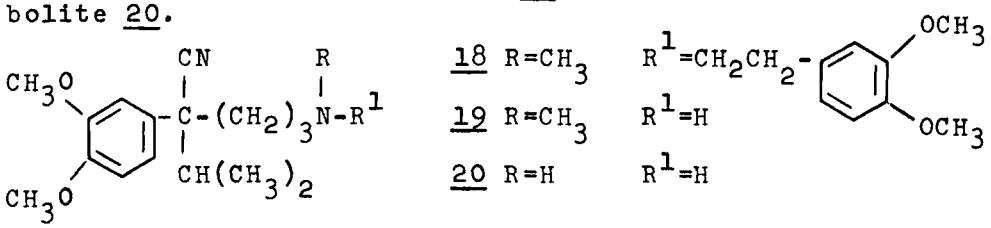


16 R=Et

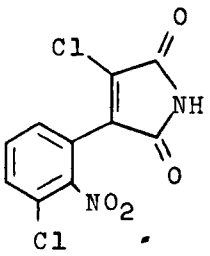
17 R=H

Methadone is metabolized in part via reduction to the symmetric methadols. α-l-Methadol and α-l-N-desmethyl methadol were identified as metabolites of d-methadone. Both of these metabolites are potent analgesics (58) and may contribute to the activity seen with d-methadone.

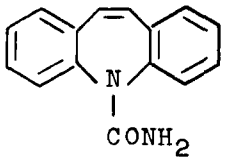
Thioxotremorine is converted to oxotremorine in vivo (59). Verapamil 18 is metabolized via a series of N- and O-dealkylations (60). In humans the active metabolite 19 was found to be two to three times more concentrated in plasma than either the parent 18 or the other major metabolite 20.



Oxidation of the pyrrole ring of pyrrolnitrin is the major route of metabolism by rat liver microsomes (61). Among the products are the highly reactive maleimide 21 and the corresponding succinimide derivative.



21



22

Metabolic fate of specific compounds - Procainamide is metabolized in man and monkey by N-acetylation. The monkey also N-dealkylates procainamide, a pathway of minor significance in man. More unchanged procainamide is excreted in man (60%) than monkey (2%) (62).

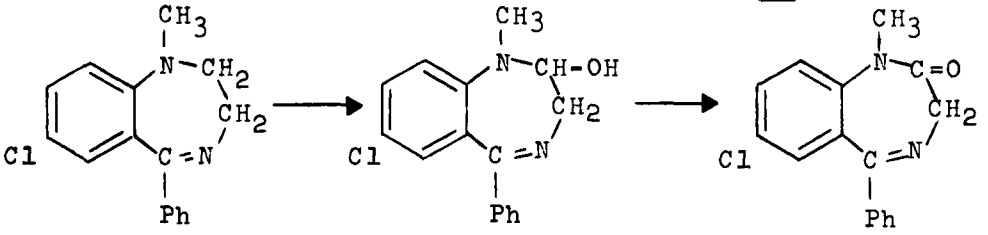
In the first four hours after dosing, indomethacin and its glucuronide were major excretion products in human urine. Later, products of N-demethylation and amide hydrolysis predominated (63).

Carbamazepine 22 is metabolized to the 10,11 epoxide, as well as 10 and 11 hydroxy derivative. Aromatic hydroxylation also occurs (64,65).

The N-t-butyl analog of chlorcyclizine was converted to norchlorcyclizine by the rat in vivo and by rat liver 9000 x g supernate. Attempts to demonstrate the unusual N-dealkylation in enzyme preparations from rhesus monkey, mouse, guinea pig, rabbit and dog were without success (66).

Cyclohexylamine is metabolized only to minor extent in man (1-2%). The only products of deamination reported were cyclohexanol and cyclohexane-1,2-diol. In rats and guinea pigs metabolism was more extensive (4-5%) and yielded a variety of alcohols and amino-alcohols (67).

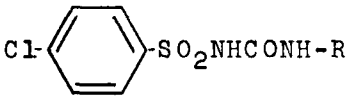
The metabolism of a number of benzodiazepines were reported, including lorazepam (68), demoxepam (69,70), medazepam (71) and oxazepam (72). In vitro oxidation of diazepam under oxygen-18 showed that the hydroxyl oxygen of the 3-hydroxy metabolite came from molecular oxygen (73). The conversion of medazepam, 23, to diazepam, 24, was found to occur via an intermediary carbinol amine, 25 (71).

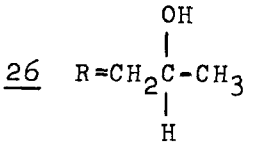


23 25 24

The in vivo metabolism of β-adrenergic blocking drugs was recently reviewed (74). Additional studies with propranolol in man have revealed a number of new metabolites: N-desisopropyl propanolol, 1-(α-naphthoxy)-2,3-propylene glycol, α-naphthylacetic acid, α-naphthol, 1,4-dihydroxynaphthalene and a ring hydroxylated derivative of 1-(α-naphthoxy)-2,3-propyleneglycol (75).

Two new human metabolites of chlorpropamide have been identified as isomeric hydroxychlorpropamides, 26 and 27 (76,77).



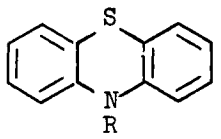


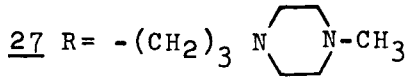
26 R=$CH_2C(OH)(H)CH_3$

27 R=$CH_2CH_2CH_2OH$

Similar metabolites involving hydroxylation of an N-propyl group have been reported for probenecid (78,79).

The phenothiazine drug, perazine, 27, is known to be metabolized by N and S oxidation, hydroxylation and N-dealkylation. A new metabolite, 28, resulting from the degradation of the piperazine ring has been found to accumulate in tissues during chronic dosing. It's half-life is much longer than 27 or its major metabolites (80,81).



27 R= $-(CH_2)_3$ 

28 R= $-(CH_2)_3 NCH_2CH_2NH_2$

A number of other drugs containing a piperazine group undergo a similar degradation (82). Most metabolite identification work in the past has concentrated on those metabolites eliminated in urine and feces. The studies above (80,81) demonstrate the desirability of examining tissues for persistent metabolites when samples can be obtained.

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Chapter 25. Prospects for Gene Therapy

Alfred G. Knudson, Jr.
Medical Genetics Center
Graduate School of Biomedical Sciences
The University of Texas
Health Science Center at Houston

Almost since the first successful genetic transformations were accomplished in bacterial systems, notice has been taken of the implications of such directed genetic change for medicine. The burden of genetic defects is so large, and the means for dealing with it so meager, that new approaches are ever welcome, especially any which may strike at the underlying cause, the abnormal genetic material itself. The current prospects for such a gene therapy constitute the subject of this review.

The first example of directed genetic change to be discovered was transformation.[1] Pneumococci of one capsular specificity were stably transformed into those of a second specificity by a principle extracted from the latter. The subsequent discovery by Avery et al.[2] that this transforming principle is DNA was of course the first demonstration that DNA is genetic material. Although much has been learned about the process in bacteria, the reports of transformation in mammalian cells have not been reproducible.[3] One identifiable obstacle is that mammalian cells contain deoxyribonucleases which degrade foreign DNA. This obstacle is circumvented by DNA viruses, which have a protective outer coat. This coat has been dissociated from the DNA of polyoma virus and used to enclose foreign (mouse) DNA to protect the latter during its delivery to human cells.[4] It therefore seems possible that the first step in transformation, delivery of intact DNA, might be feasible.

But can the appropriate DNA be delivered? In the original transformation experiments the entire bacterial DNA was extracted. Such a mass transit of DNA in man would be unduly complicating, and attempts would be required to isolate only the specific DNA required. Isolation of gene-specific DNA has been accomplished in bacteria.[5] Whether the same approach could be used in mammalian systems is another matter, but there is a conceivable indirect approach. If the messenger RNA for a specific protein can be isolated even in small amounts, it could be used as a template for synthesis of the homologous DNA, with reverse transcriptase as the catalytic agent.[6,7] If the messenger RNA were not available, knowledge of the amino acid sequence of a polypeptide chain and consideration of the genetic code might be employed to construct the appropriate artificial gene.[8]

Even if delivery of an appropriate DNA could be accomplished there remains uncertainty about the ability of the new DNA to function. One fate is that the DNA may be lost, or fail to replicate with division of the host cell. The greatest assurance would be provided by integration of the donor DNA into host cell DNA, a process which depends upon the physical state of both DNAs.[9] Of course, integration itself does not assure

the proper function of donor DNA, and special attention must be paid to its transcription and translation.

Perhaps a more promising approach to directed genetic change is transduction, a process first discovered in Salmonella.[10] Transduction involves the transport of a gene from one cell to another by a virus, a bacteriophage in the case of bacterial cells. Transduction may be general, involving several different genes, or special, involving a particular gene, as with the transduction of galactose genes of E. coli by lambda bacteriophage. Although transduction has not been demonstrated in animal cells, the first prerequisite, integration of viral DNA into host DNA, is met by tumor viruses; for DNA tumor viruses it is the viral DNA, for RNA tumor viruses it is complementary DNA which is integrated.

Not only does transduction have the advantage over transformation with respect to cell entry and maintenance of the DNA after entry but it also presents the possibility of transfering specific genes. So far there is no completely demonstrated example of integration and continuing function in mammalian systems, but three reports are of interest in this connection. Munyon et al.[11] have reported that mouse cells lacking the enzyme thymidine kinase have acquired this activity following infection with irradiated herpes simplex virus capable of inducing enzyme activity, but not following infection with a herpes mutant lacking this capacity. Merril et al.[12] have reported the completely unanticipated result that human galactosemic cells have acquired the missing transferase activity following infection with lambda bacteriophage carrying the corresponding E. coli galactose gene. A third report is that of Rogers[3] concerning an in vivo experiment in children with a severe metabolic error attributable to a deficiency of arginase. Observing that the rabbit papilloma virus has an associated arginase activity and causes reduction of blood arginine levels in man, Rogers and his physician colleagues administered the virus to the affected children. Early follow-up studies have revealed a marked lowering of blood ammonia levels. Of course it has not been demonstrated in any of these three reports that true transduction has occurred.

A new and potentially useful tool has recently been provided by Jackson et al.[13] Operating with the knowledge that tumor viruses integrate their DNA into host cell DNA, these investigators have constructed in vitro, utilizing a series of enzymatic steps, a circular DNA duplex which contains DNA from a tumor virus (SV-40) and another source (lambda bacteriophage). This procedure could be used then to transfer into mammalian cells any specific DNA which can be isolated or prepared.

An entirely different approach to directed genetic change is that of cell hybridization. The discovery[14] that irradiated Sendai virus induces fusion of animal cells has permitted a whole new approach to the mapping of human genes on specific chromosomes[15] and to the analysis of the role of specific chromosomes in cancer.[16] Relevant to the discussion

of gene therapy is the report of insertion of genes from chicken cells into mouse cells.[17] The therapeutic utility of cell hybridization remains remote, however, until selection of the properly engineered product can be accomplished, until immunologic rejection of the hybrid product can be avoided, and until transformation to the malignant state can be prevented.

Even when genetic change by these methods or by some yet unconceived method may be realized, there will remain problems before it can become a genetic therapy. One such problem is the time of treatment. Some genetic disorders, probably including most chromosomal abnormalities, are irreversibly established at birth; for them any therapy would necessarily be prenatal. Others, including many inborn errors of metabolism, develop after birth and treatment could begin soon thereafter. Another problem concerns tissue specificity; any successful treatment must alter the tissue(s) responsible for disease. For some conditions, such as phenylketonuria, it may be necessary to affect only one organ, while for others, such as most lipid and mucopolysaccharide storage diseases, it may be necessary to affect several organs. Still a third problem is that of the quantitative requirement upon any gene therapy. It would not be adequate to "cure" one cell per million. It would probably not be possible for most tissues to cause selective proliferation of one cured cell, so many cells would need to be altered. Since many enzymopathies occur in the presence of one per cent of activity, that level is a minimum requirement. For most conditions a level of 10-20 per cent of normal activity would probably be required, in view of the fact that many clinical disorders occur with that much residual activity.[18]

These problems comprise a class of positive requirements. There is also a negative requirement -- that no serious risks be taken, especially if the risks are worse than the original disease. A serious identifiable risk in the case of transduction is that of carcinogenesis. It now appears that all animal viruses which are capable of integrating their genomes into the host cells are, at least under some conditions, oncogenic.[19] Then again latent viruses of this type have been incriminated in autoimmune diseases.[20] What makes these possibilities particularly important is that a transducing virus might be passed from patient to normal individuals associated with him.

Any program of gene therapy must be weighed against other approaches to the control of hereditary disease. These measures are several and depend to a great extent upon the particular hereditary diseases in question. For purposes of this discussion it is convenient to classify genetic disorders into four categories: (1) chromosomal abnormalities such as Down's syndrome (mongoloid idiocy), (2) Mendelian conditions, including the inborn errors of metabolism, (3) polygenic disorders, such as diabetes mellitus, schizophrenia, and essential hypertension, and (4) somatic genetic disorders, such as cancer and, possibly, autoimmune diseases.

The chromosomal abnormalities make severe demands upon any therapy. Treatment would in most instances need to be instituted prenatally, it would need to affect multiple tissues, and it would need to affect a large fraction of the cells in those tissues. Conversely, it is precisely for this group of diseases that prenatal diagnosis by amniocentesis with subsequent induced abortion is most promising; in fact, concrete proposals for mass screening have already been presented.[21] Whether or not chromosomally abnormal fetuses could ever be prevented in the first place is dubious. Although only 0.5 - 1.0 per cent of all newborn infants is chromosomally abnormal, about 30 - 40 per cent of all spontaneous abortuses are so affected.[22] From this one can calculate that about five per cent of all recognized conceptuses are chromosomally abnormal, suggesting that the "cause" is some basic process with high failure rate.

Mendelian disorders may be inherited in autosomal dominant, autosomal recessive, or X-linked fashion and there are corresponding differences in the merits of various approaches to their control. Severe dominant conditions are very rare because many of those affected fail to survive to reproduce. Their incidence in a population is therefore maintained by recurrent mutation, and a large fraction of those affected present no family history which could have served as a warning and led to prevention. Many dominant conditions involve growth disorders already apparent at birth, so any treatment would again encounter great obstacles. Perhaps the best approach will be to develop means for prenatal diagnosis.

Many Mendelian recessive conditions are inborn errors of metabolism which are reversible following birth and they have understandably been the chief target of previous therapeutic efforts. Prevention is also more feasible because the unaffected heterozygous gene carriers can often be detected. Effective screening programs for such carriers could lead to identification of matings between two such carriers. One-fourth of the offspring of such matings can be expected to be affected. In some instances, as with Tay-Sachs disease, prenatal diagnosis and abortion are possible.[23] This general methodology is also applicable to X-linked disorders, although a significant fraction (up to one-third) of all affected individuals results from new mutation. Therefore a more general solution for prevention of Mendelian disorders of all kinds would be screening of all pregnancies by amniocentesis, as proposed for chromosomal abnormalities.

Any gene therapy for Mendelian disorders would necessarily compete with other therapeutic measures, and there are a few instances of effective treatment.[24] Circumvention of some metabolic defects can be accomplished by elimination of an accumulating substrate from the diet; for example, the early institution of a galactose-free diet in a galactosemic child or of a low phenylalanine diet in a phenylketonuric child may lead to normality. Circumvention can in some instances be effected by supplying a product of a reaction, as with cortisone in adrenal hyperplasia. Drugs

are sometimes effective, as with the use of penicillamine to eliminate copper in Wilson's disease. Restoration of enzyme activity has been possible in some cases of methylmalonic acidemia with vitamin B_{12} and of homocystinuria with pyridoxine; apparently the enzymatic defect in some cases involves the cofactor binding site. In bilirubin conjugation defects some alleviation of jaundice is accomplished by the administration of phenobarbital, which induces a considerable increase in the amount of a partially defective enzyme. Replacement of a defective protein is exemplified in the treatment of hemophilia or of agammaglobulinemia. Finally, treatment by tissue or organ transplantation, exemplified by kidney transplantation for hereditary polycystic disease, is in effect replacement of defective genes.

The polygenic disorders, those which result from interaction of two or more genes, are by far the most common hereditary diseases. All chromosomal and Mendelian disorders together affect about one per cent of individuals born in the United States, whereas each of diabetes mellitus, schizophrenia, and essential hypertension has a higher incidence. Prevention of these disorders is not practiced at all at the present time, largely because there is inadequate understanding of their pathogenesis. On the other hand, treatment has contributed much more, as with the use of insulin and anti-hypertensive agents. Gene therapy for these conditions seems very remote, principally because the basic gene defects are not known and because gene therapy would need to be very safe to warrant use in patients for whom there are so many other available measures.

The only well defined category of somatic genetic disease is cancer. It is now probable that environmental agents which initiate cancer do so by altering the host's genome. This change may also occur spontaneously. There is evidence that two or more steps are involved and that one may be inherited in germ cells, thereby predisposing the host to cancer.[25] These individuals may be considered along with those in other genetic categories as far as prevention and treatment are concerned. Even the cancer itself should be viewed in this light and gene therapy should not be excluded from consideration.

It is too early to say whether gene therapy will ever be added to the armamentarium against any of these categories of disease. It is an exciting and dramatic concept. It also might present new hazards which would outweigh its utility in comparison with other measures. Still, these other measures are so poorly developed that exploration of this fundamentally different approach should be continued.

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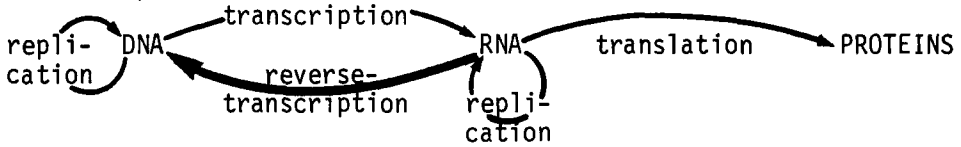
Chapter 26. Reverse Transcription and Its Inhibitors

M.A. Apple, University of California Medical School, San Francisco

I. INTRODUCTION

A. "Reverse transcription" is the name given to the process of complementary transcription from a polyribonucleotide strand into a polydeoxynucleotide. So far as is now known, the process is the same(not the reverse) of that used to transcribe a complementary strand of messenger RNA from its DNA template[1-3] or to duplicate DNA?[4,5] Reverse transcription appears so far to be simply another complementary transcription with little mechanistic uniqueness. Because of a prevailing belief that DNA in cells could only be transcribed into complementary RNA or replicated(self-transcribed)into complementary DNA, and that RNA once made could only function in protein synthesis (i.e.information transfer was unidirectional as DNA→RNA→PROTEINS)it required novel insight to discover that RNA viruses carry or produce enzymes which catalyze RNA→RNA replication[6,7] and RNA→DNA or "reverse" transcription?[8,9] Both of the latter processes were postulated as essential prior to their actual discovery since DNA→RNA information flow was inadequate to account for the reduplication of RNA viruses.



B. New Life Styles Among Viruses

It now appears that RNA viruses which carry out direct RNA→RNA replication are those with a lytic life cycle(e.g.polio virus) in which the virus attaches to a cell, enters, uncoats its RNA, replicates RNA→RNA, assembles new virus and releases new virus,killing the host cell. Alternatively, RNA viruses which contain enzymes that, under a specified set of conditions can catalyze RNA→DNA reverse transcription, have a non-lytic life cycle which includes a potentially long-lasting eclipse or latent stage; viruses of this type are mostly cancerogenic or oncogenic-RNA(abbreviated to "oncorna")viruses; the early steps are similar to other RNA viruses just described except the fourth step is RNA→DNA transcription. This new DNA product may (a)remain as free, dormant intracellular(intranuclear?)DNA, or (b) replicate with or independently of cell mitosis, or (c)transcribe into products serving to complete new virion production or (d)transcribe into products expressing information that converts normal cells into cancerous ones. If the infection is productive, new virus particles may assemble and new particles will then bud off enclosed in cell membrane envelopes(virions) without killing their host cell![10,11] This unusual life cycle poses a different set of problems than those posed by the standard viral targets of antiviral vaccines and drugs which have been previously encountered.

C. Biological Significance of Reverse Transcriptase

Among the implications of RNA→DNA transcription are:(a)that there is an oncorna viral enzyme catalyzing it which has unique properties, and selective blockade of this enzyme serving as a drug receptor could(i)prevent oncorna virion progeny production or(ii)prevent oncogenic viral transformation of normal cells into cancer, (b) that any new RNA carried into cells could

have its information content carried permanently as new DNA gene information, (c)that this transcription may provide a mechanism for the process of gene amplification[12-14] by which a small portion of the cell's DNA information is reduplicated extensively, possibly from an RNA copy of that portion of the original DNA, (d)that the DNA copy can help explain the long term eclipse and later reexpression of oncorna viruses or their RNA information[15,16] in infected cells, (e)that the oncorna viral enzyme catalyzing reverse transcription may serve as a possible marker for oncogenic RNA viruses and/or cancer cells under a variety of diagnostic or prognostic circumstances;[17] and (f)that purified reverse transcriptases can be used to make pure DNA gene copies of available messenger RNAs for various applications including specific gene transplantation.[18-20]

D. Polymerase Activities of Reverse Transcriptase

There has been a lack of clarification of whether the RNA►DNA biocatalyst is specifically associated only with oncorna viruses and cancer cells or is a more generally occurring biophenomenon. Further complicating this analysis is the failure to develop generally acceptable criteria for defining reverse transcriptase that uniquely distinguishes it from other polymerases.

It is probable that two or more types of enzymes can catalyze RNA►DNA reactions: (a) a variety of similar but immunologically distinguishable viral enzymes which accept polyriboadenylate:oligothymidylate as an early optimal substrate, found only in virions of the oncorna-type viruses;[22,23] (b) one of several animal cellular DNA►DNA polymerases which accept a variety of double-stranded RNAs or RNA:DNA duplexes as non-optimal substrates[24-26] and one or more bacterial cell enzymes such as the DNA►DNA repair enzyme of Eschericia which accepts RNA templates as non-optimal substrates.[27,28] The relative rate at which enzymic synthesis of DNA occurs using RNA as a template as compared to DNA as a template serves as a hallmark for finding the real reverse transcriptase among enzymes from different sources; e.g. for oncorna virus the rates are 50-500%, bacterial enzyme rates are<1%, invertebrates<1% and primates<1%.

ROUGH COMPARISON OF ACTIVITIES OF DIFFERENT DNA POLYMERASES(units/mgm)

Template/substrate	oncorna virus	bacterium	invertebrate	human
poly-rA	0	0	0	0
poly-rA:oligo-dT	800	100	15	50
poly-d(A-T)	200	10000+	1000	400
nicked DNA	200	10000+	1000	500
RNA	400	100	50	5

This table of very approximate estimates of relative DNA synthesis rates indicates that any DNA polymerase could show some measurable "reverse transcribing" activity;[21] but only in viruses is it the major preference.

E. Inhibitors: To Be or Not To Be, The Assay is the Question

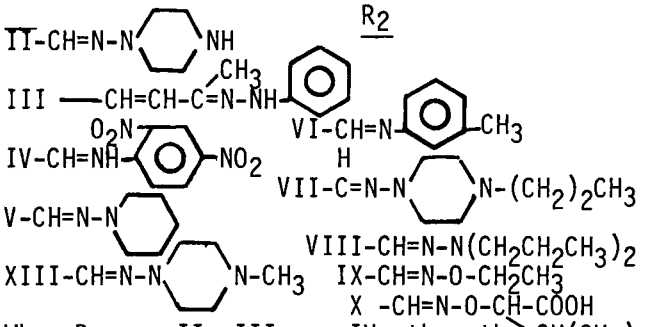
Analysis of the assay conditions in vitro generally employed for reverse transcriptase determination reveals a variety of standard substrates and of interacting substances which influence observed rates of catalysis or measured inhibitor/activator effects;[29-31] e.g., detergents[32] increase catalytic rate and affect activities of ansa-macrolides; cations alter catalytic rate (Mg++,Mn++)differently with different substrates and are of course themselves influenced by a variety of chelators or cation binding buffering agents;[33] thiols react with some potential inhibitors or otherwise influence

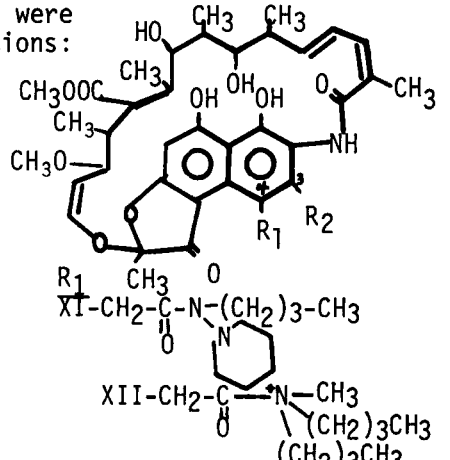
measured activities.[34] These assay condition problems account for much misinterpretation of relative activities or inhibitions and non-reproducibility between laboratories now encountered. Reverse transcriptase assay conditions employed by twenty laboratories studying the enzyme showed these ranges: 0.01-0.10M Tris(hydroxymethyl)aminomethane·HCl buffer at pH 7.8-8.6, 0.5-20mM $MgCl_2$, 0-0.120mM KCl or NaCl, 0.2-100mM dithiothreitol (or mercaptoethanol or reduced glutathione), 0.005-0.5% nonidet P-40 or triton X-100 and 0.2-2mM ethylenediaminetetraacetic acid with a variety of templates, the most common of which is polyriboadenylate:oligodeoxythymidylate, at temperatures from 30°-41°C for periods of time from 10 to 240 minutes.

II. CLASSES OF MOLECULES WHICH BLOCK REVERSE TRANSCRIPTASES IN VITRO

A. Ansamacrolides

(1) Rifamycins have been reported as inhibitors of poxvirus (a DNA virus) maturation along with other ansamacrolides, the streptovaricins.[35] Inhibition of mammalian viruses growth and blockade of oncorna virus induced cancerous transformation in vitro by rifamycins[36] were reported in 1969 and cellular reverse transcription inhibition was reported in 1970.[37] Actions of the rifamycins have been reviewed.[38] A major mode of action in cells of newer derivatives is blockade of DNA→RNA polymerases.[39,40] Most derivatives studied on DNA polymerase or on reverse transcription were substituted on one of the two indicated positions:



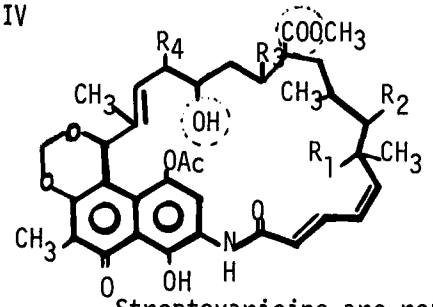


When R_2 was II, III, or IV, then the rifamycin SV derivative was reported to be a selective inhibitor of viral reverse transcriptase.[41] If the R_2 was V-X or the R_1 was XI or XII then the derivatives could fully inhibit purified leukemia cell DNA polymerases without selectivity for viral reverse transcriptase.[41] Most derivatives in which R_2 was -CH=N-NH-R_3 where R_3 was either a simple aliphatic chain or ring were very potent inhibitors of murine sarcoma virus reverse transcription.[42] The stability, protein binding, solubility, partitioning, comparative activity, absorption, distribution, elimination and toxicity of rifamycins of clinical interest have been reviewed.[43,44] Rifampicin (R_2 is XIII), is reported to suppress cancer formation induced by adenovirus-12 (a DNA virus) in hamsters,[45] but not by a RNA tumor virus in mice.[46] XIII binds specifically to E.coli DNA→RNA polymerase[47] and can be cytotoxic while not blocking Rous Sarcoma Virus-induced cancerous transformation or new oncorna virus production.[48]

(2) Streptovaricins

The structure and many chemical properties of this group of ansamacrolides have been studied,[49,50] and a review of other properties pertinent to their action on reverse transcriptase will soon be available.[51] A mixture of naturally occurring compounds called streptovaricin complex is an inhibitor of E. coli RNA (but not DNA) synthesis[52] and an inhibitor of E. coli

XIV



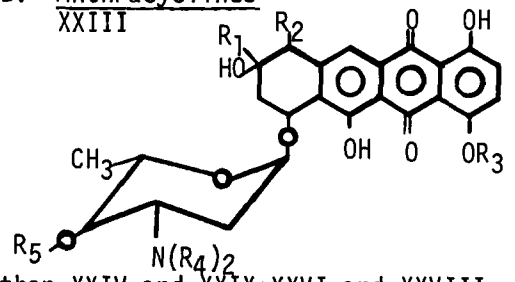
DNA→RNA polymerase. The complex consists of[49] at least streptovaricins A (R_1, R_2, R_3, and R_4 are respectively OH, OH, Ac, OH)XV; B (H, OH, Ac, OH)XVI; C(H, OH, H, OH) XVII; D (H, OH, H, H)XVIII; E(H, =O, H, OH)XIX and G(OH, OH, H, OH)XX. F is the lactone (XXI) corresponding to XX with the involved groups indicated in dotted circles.

Streptovaricins are reported to inhibit murine leukemia viral reverse transcription.[53] Cancerous transformation by Maloney Sarcoma virus in vitro was reported inhibited by streptovaricin-D but not by A.[54] Streptovaricin complex is reported by one group to be composed of inactive components A, E and F and weakly active B and C and very active MLV reverse transcription inhibitory D and G;[55] while another group reported A, C, D and C-triacetate XXII are all inactive against MLV.[42]

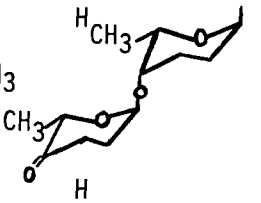
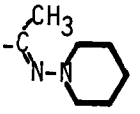
Geldanamycins and tolypomycins are also ansamacrolides with reverse transcription and DNA→RNA polymerase inhibiting activities.[56]

B. Anthracyclines

XXIII



Anthracycline chemistry has been recently reviewed.[57,58] A number of natural products of this group can intercalate into DNA.[59,60] XXIV and XXV have a clearly clinically useful anticancer effect in man.[61,62] XXIV, XXV, XXVI, XXVII and XXVIII but not XXIX block oncorna viral reverse transcriptase, and XXV-XXVIII are more potent than XXIV and XXIX;XXVI and XXVIII prevent oncorna virus replication in tissue culture.[63] XXVIII also blocks vesicular stomatitis virus reproduction in vitro (VSV is a non-oncorna RNA virus).[64]

		R_1	R_2	R_3	R_4	R_5
XXIV	Daunorubicin	$-C(=O)-CH_3$	H	CH_3	H	H
XXV	Adriamycin	$-C(=O)-CH_2OH$	H	CH_3	H	H
XXVI	Cinerubin	$-CH_2CH_3$	$-C(=O)-O-CH_3$	H	CH_3	
XXVII	Piperazino-Daunomycin		H	CH_3	H	H
XXVIII	Acetyladriamycin	$-C(=O)-CH_2O-C(=O)-CH_3$	H	CH_3	H	H
XXIX	N-acetyldaunomycin	$-C(=O)-CH_3$	H	CH_3	$-C(=O)-CH_3$	H

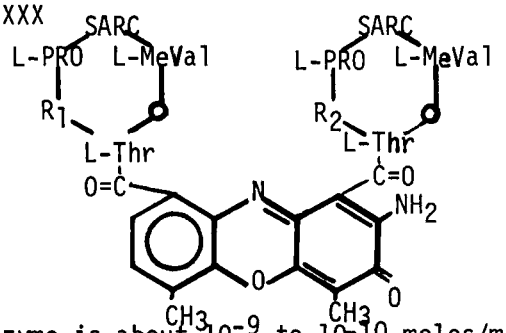
The largest structure-activity studies of derivatives of this series[57,65] suggested that most R_4 derivatives, which in molecular models act to block the amino group's bonding to a polymerase polynucleotide substrate phosphate are relatively ineffective as DNA polymerase inhibitors o tumor inhibitors. Alternatively XXVI is more active than XXIV, and R_1 variants of XXV in which

the R_1 group appears in molecular models to be directed at the enzyme of the enzyme-substrate complex, maintain or improve their inhibitory activities and block oncornavirus reverse transcription at concentrations around 10^{-8} moles/ml or less.[63,66]

C. Cactinomycins

The mechanisms by which these drugs work has been considered[67] to be via intercalation of the phenoxazone chromophore into a double-stranded polynucleotide structure along with an anchoring due to its polypeptide loops extending in opposite directions[68,69] (not in the same direction as proposed earlier[59]) blocking further polynucleotide synthesis.[70,71]

XXX

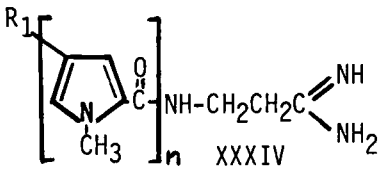


Derivative	R_1/R_2 peptide component
C_1/D	both D-valine XXXI
C_2	D-valine, D-alloIsoleucine XXXII
C_3	both D-alloIsoleucine XXXIII

It has been reported that C_3 is more inhibitory to the reverse transcriptase of Rous sarcoma virus than the other two,[72] and that the in vitro concentration required to block this enzyme is about 10^{-9} to 10^{-10} moles/ml. Thus C_3 is the most potent inhibitor per mole of any reported drug acting on oncorna virus reverse transcription, although it is relatively inactive on the Kornberg DNA→DNA enzyme.

D. Polypeptides

(1) Distamycins



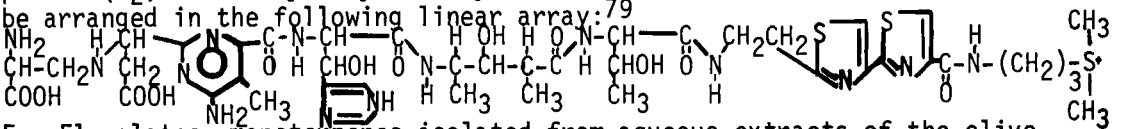
XXXIV

R_1	#	n
-HN-CHO	XXXV	3
-HN-CHO	XXXVI	5
-HN-C(=O)-	XXXVII	2
-HN-CHO	XXXVIII	2
-HN-C(=NH)-CH_2-NH-C(=NH)-NH_2	XXXIX	2

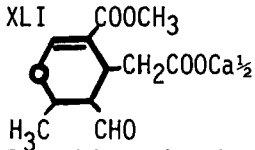
The antiviral activity per mole of XXXVIII is about 1/7 of that of XXXV, while XXXVI is about 16-fold more active than XXXV.[73] XXXVI inhibits Friend Leukemia virus and Maloney sarcoma virus reverse transcription by half at 20 µg/ml.[46] XXXVII is at least 3-fold less potent an inhibitor of Rous sarcoma virus reverse transcription than XXXVI.[73] The binding of XXXVI to polynucleotides appears to affect enzyme attachment of DNA→RNA polymerase to DNA and instantly stops initiation of new RNA chains, while chain elongation is resistant to the action of the drug.[74,75]

(2) Bleomycins

The bleomycin complex[76] appears to inhibit murine leukemia virus reverse transcriptase but not mouse lymphoma DNA→RNA polymerase.[77] One component (A_2) can be hydrolyzed to yield 7 different amino acids,[78] which might be arranged in the following linear array:[79]

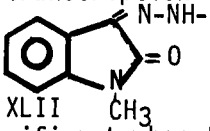


E. Elenolates, monoterpenes isolated from aqueous extracts of the olive plant Olea sp. are general virucides in vitro,[80,81] a property lost if eleno-

XLI 

late is preincubated with cystine, histidine or lysine, while isoleucine, leucine and tyrosine showed no effect.[80] XLI is reported to selectively inhibit murine leukemia virus reverse transcriptase but not E. coli polymerases.[82]

F. Thiosemicarbazones were the first significant antiviral drugs[83] and when N-methyl-isatin-thiosemicarbazone produced dramatic results as a prophylactic agent for smallpox[84] the era of drug-induced antiviral prophylaxis was truly initiated. Aromatic thiosemicarbazones appear to inhibit viral reverse transcription in vitro.[85] XLII works intracellularly on DNA viruses and appears to block either DNA→RNA synthesis or the function of the RNA transcribed product.[86,87] The reverse transcriptase inhibiting activities reported recently for drugs of this group are not as significant when the viral enzyme activity is optimized by addition of thiols and represent an inhibition measured under sub-optimal assay conditions.[34,85]



XLII

G. Propanals

A number of drugs of this group of simple carbohydrates are relatively non-toxic anti-cancer agents in mice[88,89] and are readily reactive with the base-pairing region of the guanine of polynucleotides which could account for their in vitro inhibition of the Rous sarcoma virus reverse transcription assay.[90,91] Others: The following compounds have been reported to inhibit viral reverse transcriptase: congo red,[92] axenomycin,[93] ethidium bromide,[93] cytosine arabinosidetriphosphate,[94] single-stranded polynucleotides,[95] and lycorin alkaloids.[111]

III. COMPARISONS OF REVERSE TRANSCRIPTASE INHIBITORS

A. Relative Inhibition of RNA→DNA - vs $\frac{\text{DNA→DNA}}{\text{DNA→RNA}}$

Of the drug groups showing significant activity on viral reverse transcription, for some members of the rifamycins, streptovaricins, cactinomycins, distamycins and thiosemicarbazones there is evidence supporting the thesis that their major mode of antiviral action is through blockade of normal cellular DNA→RNA transcription.[47,86,87,96-100] It is thus rather inexplicable that so much emphasis has been placed on developing selective reverse transcription inhibitors that do not inhibit DNA→DNA replication when it appears that this replication may not be the major obstacle, but virtually no effort has been spent on discovering or developing reverse transcription inhibitors that do not also inhibit "forward" transcription from DNA→RNA.

B. Relative Potency Scale

Based on the most active reported members of each series discussed, without regard to selectivity of action, the potency on a per mole basis of the reported reverse transcription inhibitors varies over a 10,000-fold range in vitro, suggesting an approximate potency scale in which cactinomycins > anthracyclines >> distamycins ≥ rifamycins > elenolates ≥ bleomycins ≥ streptonigrins > thiosemicarbazones > propanals > streptovaricins. This does not predict which group will ultimately produce the best in vivo drugs since so many other factors are involved.

C. Prospects for Potent and Selective Inhibitors: unique opportunity. Although it was only recently discovered, the process of reverse transcription offers a historically unique new target for drug design. Either the viral

enzyme, or the substrate of the enzyme-substrate complex can already by prepared in large quantities in fairly pure form with ease by currently available techniques.[101] It is reasonable to presume that the enzyme offers a more potentially selective target than the substrate, but that drugs aimed solely at the process of transcription would not, unless new data emerges, distinguish"forward" from "reverse" transcription. An optimal drug might block the E-S complex formation/action by being simultaneously able to recognize either the enzyme or the polynucleotide substrate as its receptor. The design of such new drugs is clearly possible now.

IV. REVERSE TRANSCRIPTASE INHIBITORS: RELATIVE BIOLOGICAL EFFECTS

A. Methods of Study and Method Critiques

Assay methods for new oncorna virus progeny production are subject to errors when drugs inhibiting virus production at an early replicative step are studied since viruses whose synthesis was past the point of drug action when drug was administered will become functional viral particles in the presence of the drug. Drugs shutting down cellular processes to the point of producing a delayed death or replicative failure of cells may give the false impression of specific antiviral activity in vitro. Assays requiring the progeny virus to be active to be counted in vitro as plaques or foci are subject to virus being inactivated by a high extracellular drug concentration without any real selective activity in infected cells as well as spurious cytotoxic effects. An antitransforming activity in vitro using a focus assay must be under conditions allowing normal cellular proliferation, to be valid. Preventing viral cancer formation in vivo requires that drugs be given after virus penetration of cells but before the earliest transformed cells begin proliferation so that antiproliferative agents will not also give the false impression of also being antiviral. A good in vivo assay should also measure actual cancer development;e.g.,pre-leukemic splenomegaly inhibition can be induced by non-antiviral cytotoxic drugs.

B. Blocking New Virion Progeny Production

Only a few of the drugs reported as reverse transcription inhibitors have so far been found to block production of new progeny virions of oncorna virus. These include streptovaricins,[56] cactinomycins,[102] anthracyclines,[102] and rifamycins[103] but some observations are subject to the above criticisms. Observations of progeny production blockade strengthen the hypothesis that RNA→DNA synthesis is a *sine qua non* of oncorna virus reproduction, but do not assure it since these agents might affect some other polynucleotide transcriptions to some degree as well.

C. Cancerous Transformation

The property of contact inhibition of normal cell movement and growth is so diminished by their transformation into cancer cells that discrete colonies(foci)of piled up cells appear and indicate the transformation event.[104] The hypothesis that reverse transcriptase inhibitors might block oncorna-virus-induced in vitro oncogenic transformation has apparently been verified using the focus assay with several groups of drugs including streptovaricins,[105] cactinomycins,[102] anthracyclines,[102] rifamycins[48] and distamycins.[107] Any activity of the reverse transcriptase inhibiting drugs which prevents cells from forming discrete foci will be measured by this procedure, but it is very rapid (< 1 week) and a reliable indicator of the viral oncogenic events.

D. In-vivo Chemoprophylaxis of Cancer: A New Frontier

Among the ultimate uses of reverse transcription inhibitors is the possible chemoprophylaxis against oncorna virus cancers.[107] These drug agents show promise in this acid test of their effectiveness: streptovaricins are reported to block pre-leukemic splenomegaly in oncorna virus infected mice[108] and only one injection of either cactinomycins or anthracyclines can actually prevent RSV-induced avain sarcoma development.[102]

E. Reverse Transcriptase Inhibitors as Cancer Chemotherapeutics

There is no a priori reason to expect reverse transcriptase inhibitors to be therapeutic in established cancers unless this enzyme function is needed to maintain the transformed state as well as to initiate it. Since two of the most potent groups of viral reverse transcriptase inhibitors, cactinomycins and anthracyclines, each have examples with notable clinical effects (e.g., dactinomycin and adriamycin are occasionally curative in human cancer) and animal model studies show other groups of reverse transcriptase inhibitors to have some therapeutic effect, perhaps either the maintenance hypothesis deserves more exploration, or the clinically effective drugs are those which also act to inhibit DNA-directed transcriptions.

F. Reverse Transcriptase Inhibitors and Gene Amplification/Antibody Synthesis

Reverse transcription has been implicated in embryo gene amplification[103,109] and possibly antibody synthesis.[110] If this role of reverse transcriptase is further documented, some of these new drugs might prove useful for such activities as blocking the response to a particular new antigen without creating generalized immunosuppression, which would be an important advance in the organ/tissue transplant field.

V. CONCLUSIONS

Reverse transcription is a newly discovered process of enzyme catalyzed information transfer from RNA to DNA with a variety of potentially significant biological implications in virus life cycles, cancer etiology and prediction, gene amplification, embryo differentiation, response to antigens, and gene transplantation. Chemicals blocking the reverse transcription process serve not only as probes into its significance, but also as drugs with the power to prevent selected types of in vivo virus-induced cancer, virus replication, and antibody production in animal model studies. More selective and specific drugs which act as reverse transcriptase inhibitors need to be developed and a variety of structure classes of compounds so far studied have provided important leads.

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Chapter 27. Drug Receptors

Jasjit S. Bindra
Pfizer Inc., Groton, Connecticut

Various aspects of drug receptors and drug action have been reviewed in previous annual reports of this series by Bloom[1,2] and Mautner.[3] This report focuses on the several major advances that took place in this area since 1969, particularly with regard to the cholinergic receptor.

Several monographs[4-6] and proceedings of symposia[7-10] on drug action and receptors have appeared during the last few years. Of special interest are the volume by Triggle on neurotransmitter-receptor interactions,[4] and the much awaited series on drug design edited by Ariens.[7] The reader is also directed to an engaging discussion on receptor isolation and characterization in the proceedings of a recent Ciba Foundation Symposium.[9]

Conformation of Drugs and Structure-Activity Relationships (SAR): The problem of structure and conformation of drugs has been reviewed.[11-13] Portoghese, in a comprehensive review has focussed on the role of stereochemical factors in the action of drugs on excitable tissue.[13] The question whether drugs engage the receptor in a preferred "pharmacophoric" conformation[14] is still unresolved, and has led to the synthesis of conformationally restricted and rigid analogs of neurotransmitters.[15-19] Burger has discussed the information that can be gained from SAR studies concerning the complementary receptor sites.[20] Application of Hammett substituent constants and the use of molecular orbital methods in drug design are reviewed in Chapter 32.

Cyclic AMP and GMP: The relationship between hormone receptors and adenyl cyclase continues to draw attention.[21,22] It is all but certain that the adrenergic agonists act by catalyzing adenyl cyclase, and any general theory seeking to explain their action at a molecular level must be compatible with visualization of the receptor as an integral part of this enzyme system. Some recent data suggests that cyclic GMP may be involved in cholinergic neurotransmission.[23]

Receptor Theory: Neurotransmitters exert their effect on postsynaptic cells by interacting with specific sites or receptors on the excitable membrane. This interaction initiates and controls the permeability changes permitting ion movements and amplification of the electrical signal during propagation of nerve impulses.[24,25] Although the precise molecular mechanism of ion-conductance is still a matter of speculation[26], there is good evidence that binding of the agonist induces a conformational change in the receptor, thus indirectly causing a change in ionic permeability of the cell membrane. An important characteristic of drug-receptor interaction is that not all agonists are equally effective in causing this permeability change. Different drugs are capable of producing an equal biological response when occupying different fractions of the receptor pool, an observation which led to the concept of "intrinsic activity" or

efficacy, itself purely an operational term.

Implicit in several of the recent theories of drug action is the view that activation of the receptor represents a quantal rather than a graded transition. Efficacy is thus quantized and is interpreted as a measure of the probability that a receptor will assume an active configuration as opposed to an infinitely variable range of states of the receptor ranging from high to zero efficacy. This concept of quantized efficacy was the most important theoretical point emerging from Paton's original rate theory[27] and the modified dissociation rate theory.[28] Although experimental evidence for the theory is at present inconclusive,[32,29] the idea of a quantized kinetic approach continues to be developed.

Recently, Rang and Ritter noted that affinity of receptors for certain antagonists increases when applied in the presence of agonists.[30] This led to the suggestion that the agonist causes the receptor to change to a desensitized state, and that the desensitized receptor has a greater affinity for antagonists. Evidence was presented that this change in affinity is due to a conformational change in the receptor (called the metaphilic effect) brought about by the action of agonists but not of antagonists. Results of a study of the kinetics of this phenomenon were consistent with the cyclic model for desensitization originally proposed by Katz and Thesleff,[31] but with two rate-limiting irreversible steps, $AR \xrightarrow{Kd} AR'$ (densitization) and $R' \xrightarrow{Kr} R$ (recovery). The first of these is related to receptor activation, so that K_d becomes a measure of efficacy of the drug.[32] A good correlation has been observed between desensitization and the metaphilic effect at cholinergic receptors, confirming that a class of antagonists preferentially binds to the desensitized receptor.[33] The autoinhibitory effect of acetylcholine (ACh) binding to Torpedo electroplax, observed at high concentrations of the substrate, is probably directly related to the physiological desensitization phenomenon.[34]

Another theory that involves the quantal approach is based on Monod's model for allosteric properties of regulatory enzymes.[35] The extension of this model to drug action was proposed by Karlin[36] and by Changeux,[37] who postulated that the receptor macromolecule exists in two conformations or states which are in equilibrium, viz., an active state associated with increased permeability and an inactive state, regardless of whether or not a drug molecule is bound to the receptor. Drugs act as agonists or antagonists according to their selective affinity for the two conformations, and efficacy is simply a measure of its relative affinity for the two states. Partial agonists such as decamethonium show affinity for both forms of the receptor. According to Changeux and his colleagues[38-40] the macromolecular receptor for ACh is distinct from an ionophore responsible for the translocation of specific cations through the membrane. The complex, ACh-receptor together with ACh-ionophore, is regarded as the elementary structural unit of the membrane which accounts for the response to the neurotransmitter and is termed as the ACh-protomer. Evidence in favor of the allosteric theory is centered around the sigmoid nature of

dose-response curves observed for several depolarizing agents acting on the electroplax cells. When small amounts of decamethonium are present, the sigmoid character of the dose-response curves is lost.[41] This phenomenon is strongly reminiscent of allosteric effects seen with regulatory enzymes. However, although the allosteric theory is quite attractive, other alternatives could explain the observed sigmoid shape of concentration-effect curves. And as Rang[42] points out, unfortunately neither of the pieces of evidence brought thus far to bear upon the distinction between the conventional occupation theory and the allosteric theory of drug action is as yet conclusive.

Isolation of Receptors

There have been numerous attempts to detect the binding of drugs to receptors and to isolate the receptor material, and the earlier attempts at receptor isolation have been reviewed.[43,46]

There are certain obvious similarities between the problem of isolation of receptor material and that of isolating and identifying the components of the active sites of enzymes.[46] However, the problem of enzyme isolation is probably far simpler since activity of enzymes can be monitored *in vitro* by following the catalytic activity of the various fractions that are isolated. During receptor isolation, on the other hand, one is faced with the more formidable problem of identifying a system whose physiological activity is dependent upon the integrity of the cellular system and its membrane components; once a tissue has been homogenized or otherwise disrupted, its physiological responses are no longer present. For want of a better biochemical index the investigator must therefore search for macromolecules that bind correct ligands with appropriate affinity and reversibility, and that are present only in the appropriate amount in appropriate tissues.

Techniques of Receptor Isolation: Two techniques have been used to monitor AChR isolation. The use of reversible binding technique which is conceptually most attractive for receptor isolation is attended by the danger that protein denaturation or disaggregation of the receptor mosaic during extraction procedures may sufficiently alter the binding characteristics as to interfere with the recognition of it by means of techniques such as equilibrium dialysis. Alternatively, affinity labels may be used to detect and identify the receptor *in vitro*. Even though this approach results in irreversible inactivation of the receptor, the receptors can be labelled in the intact tissue and isolation of the stable radioactive complex can then be monitored by standard biochemical techniques. Thus, until more information is available concerning receptor structure and function there are advantages in using the "affinity label technique". Isolation of a functionally pure receptor must eventually involve the application of a reversible binding technique, and if the theoretical potential of such an approach is realized it would permit isolation of the crystalline drug-receptor complex which could then be subjected to X-ray procedures successfully applied to other enzyme complexes. The merits of each approach to the quantitation and isolation of pharmacological re-

ceptors have been discussed in detail by Moran and Triggle[46] and O'Brien et al.[47]

Affinity Labeling: The methodology and technique of affinity labeling of protein active sites has been reviewed by Singer.[48] Affinity labeling can be used to label not only the catalytic active sites of enzymes, but also allosteric or regulatory active sites, antibody active sites, or any other active sites exhibiting binding specificity but which do not contain any unusually reactive groupings. The method is thus potentially useful in the study of molecular properties of drug receptors. In combination with techniques of differential labeling to enhance specific site labeling, it provides a powerful tool for labeling and identification of drug-receptor macromolecules so as to permit their ultimate isolation and characterization. The method is also useful to attach fluorescent[49] or spin labels[50] to active sites as probes to study changes that may accompany various physiological perturbations of intact membrane-bound specific receptors. The use of photolysable reagents for affinity labeling which was first described by Westheimer[51] continues to be used for investigation of active sites of soluble proteins and antibodies. Recently Singer et al. used photoreactive aryl azides for affinity labeling of specific ACh sites on membranes.[52] The specificity of these reagents, however, is by no means fully established.

Receptor Isolation by Irreversible Labeling: The best known receptor alkylating agents are the catecholamine antagonists related to dibenamine which are also irreversible blockers of AChR. Several attempts have been made to label receptors with these drugs,[53] but in general it seems that the specificity of these drugs is low, so that non-specific binding is a serious problem. Various procedures with reversible protecting agents (e.g atropine sulfate) which were devised to improve specificity gave rather disappointing results.[54]

Recently Karlin and his co-workers devised a two-step procedure for obtaining relatively specific covalent labeling of AChR by the quaternary ammonium compound 4-maleimido benzyl trimethylammonium (MBTA).[55,56] The labeling compounds were applied after reduction of disulphide bridges in the electroplax to free sulfhydryl residues by dithiothreitol (DTT). Binding could be reduced by the competitive antagonist hexamethonium, as well as by the sulfhydryl reoxidizing agent cholinedisulfide. Presumably MBTA binds to the anionic subsite of AChR with the quaternary ammonium group while its maleimide group alkylates a nearby sulfhydryl group. Using tritiated MBTA to label the reduced receptor in single intact electroplax, Karlin et al. solublized the receptor and separated it from non-specifically labeled components by gel electrophoresis. The specific component of the labeling accounted for at best between 20 to 60 per cent of the total labeling and a range of 26 to 75 x 10^{-14} moles of receptors was calculated per cell.[56] This value is similar to the calculated amount of acetylcholinesterase in the cell. Purification of the solubilized receptor material by polyacrylamide gel electrophoresis in sodium dodecyl sulfate gave a single polypeptide of molecular weight 42,000 having properties previously inferred for the receptor.[57] It is likely that this

specifically labeled polypeptide component is either the receptor or a receptor subunit containing all or parts of the ACh-binding site.

Other potent affinity labeling compounds that act on the reduced cell were designed by Silman and Karlin.[58] Affinity labels designed to react strongly with cholinergic receptors in the unreduced state are p-(trimethylammonium)benzene diazonium fluoborate (TDF),[59] benzilylcholine mustard (BCM),[60] dinaphthyl-decamethonium mustard (DDM)[30,32] and 2-halogenoethylamine.[45] The uptake of ^{3}H-BCM by smooth muscle appears to be a good deal more specific than that of MBTA by the electroplax and it appears that the membrane protein which binds BCM consist largely of muscarinic receptors. DDM acts irreversibly as an antagonist of the motor endplate, and has the interesting property that it exhibits increased affinity for desensitized receptors.[32,33]

α-Bungaratoxin, a basic polypeptide of molecular weight 8,000 isolated from the venom of the snake, Bungarus multicinctus, has an irreversible curare-like action on the postsynaptic membrane of vertebrate neuromuscular junction.[61,62] These toxins also block depolarization of homologous synapses in electric tissue of Torpedo, Raja and Electrophorous and of skeletal muscle. This effect could be prevented by d-tubocurarine, which is a specific but reversible antagonist of cholinergic receptors, indicating that α-bungaratoxin interacted with the cholinergic receptors. The specificity of action of the toxin has been confirmed by several workers.[63-65]

The extraordinary specificity of bungaratoxin has been exploited for isolation and purification of the cholinergic receptor protein.[66] These workers prepared radioactive labeled bungaratoxin of very high specific activity by iodination with ^{131}I, and studied its binding to a protein component of cell membranes from Torpedo electric organs, which they solubilized by treatment with a detergent. The toxin-receptor complex was purified by chromatography and density gradient centrifugation, which clearly separated it from AChE.[67] The receptor protein seemed to consist of a tetrameric form of subunits of molecular weight about 80,000, and the number of bungaratoxin binding sites was approximately equal to the number of AChE catalytic sites in the Torpedo electric tissue. These results are in good agreement with results found by others using equilibrium dialysis methods with reversibly binding ligands.[47] Evidence that the α-BGT binding material is the receptor protein is based mainly on the finding that rate of binding of labeled toxin in membrane suspensions was decreased by preaddition of d-tubocurarine or carbamylcholine, whereas it was unaffected by physostigmine which is an inhibitor of AChE.[66] It must be noted that only a preliminary purification of the receptor has been achieved thus far. Moreover, there is evidence from work with muscles that about 15% of the toxin is bound non-specifically and only half of the toxin binding sites in frog muscle appear to be protected by curare.[68] It is therefore possible that there are two or more kinds of sites which bind the α-toxin.

Receptor Isolation by Reversible Labeling: Recently O'Brien and his colleagues isolated a particulate fraction from the electric organ of torpedo electroplax which exhibits several of the characteristics of AChR.[47,69,70] These authors presented evidence that muscarone, which is a stable cholinomimetic, was bound to the particulate fraction in the way that would be expected for binding to an ACh receptor in terms of affinity.[69] All of the compounds known to act on cholinergic receptors reversibly inhibited the binding of tritiated muscarone, whereas a wide range of noncholinergic drugs did not. Moreover, there was a good correlation between the potency of substances as agonists and their ability to inhibit muscarone binding. Using equilibrium dialysis, the Eldefrawi's and O'Brien have measured the affinity of several other cholinergic ligands to subcellular preparations of electric tissue and house-fly brain.[71] In the brain of the housefly the binding macromolecules were proteins and exhibited both nicotinic and muscarinic characteristics, whereas in the electroplax these macromolecules were phospholipoproteins endowed with nicotinic binding characteristics alone. When binding was measured over a restricted range of ligand concentration (0.1-1μM), only a single binding site was detected for each ligand; but when the concentration was extended (0.001-100μM), multiple sites for reversible binding of various ligands were revealed, two each for muscarone, nicotine and DMTC and three for decamethonium.[72] The two agonists muscarone and nicotine each showed a low affinity binding of approximately 0.5 nmole/g and a high affinity binding of approximately 0.1 nmole/g. The effect of hydrolases and the antagonism of binding by other cholinergic ligands suggests that binding is to two different sites which exhibit binding properties similar to AChR, and that both sites are on the same macromolecule.[73]

Eldefrawi *et al.* studied binding of ^{3}H-ACh after organophosphates had been used to irreversibly inhibit all AChE present in electric tissue of *Torpedo*. Two affinity sites (K_1 = 8 nM and K_2 = 68 nM) bound ACh reversibly and binding was blocked by nicotinic drugs.[74] Characteristics and concentrations of these sites were similar to those binding muscarone and nicotine, indicating that all three ligands are bound by the same two sites. In further studies these authors have examined properties of lubrol-solubilized AChR from *Torpedo* electroplax and separated the ACh-binding macromolecules by ultrafiltration and gel chromatography.[75] Only a partial purification and characterization of the AChR has been achieved thus far, but improved techniques are expected to overcome this impasse.[76]

Changeux and his colleagues[77,78] found that ultracentrifugation of crude homogenates of electric organs of *Electrophorus* in sucrose gradient afforded two classes of membrane fragments, one of which is rich in AChE and presumably originates from the innervated membrane of the electroplax. These membrane fragments form closed vesicles or microsacs *in vitro* (approximately 0.3 μm in diameter) which respond to cholinergic ligands by a change in selective permeability to cations. The rate of efflux of $^{22}Na^{+}$ from preloaded microsacs was markedly increased by cholinergic agonists carbachol and decamethonium and blocked by d-tubocurarine. Dose-response curves constructed by measuring flux data at increasing concentration of cholinergic ligands were identical with the curves obtained from electro-

physiological experiments on the monocellular electroplax.[78,79]

Evidence that much of the decamethonium was indeed bound to the cholinergic receptor site comes from the fact that α-bungaratoxin, which specifically blocks cholinergic receptors but has no effect on acetylcholinesterase, blocked the binding of radioactive decamethonium to the microsacs to the extent of 72%. Saturating levels of d-tubocurarine displace bound decamethonium to almost the same extent, and the residual 28% is bound presumably to the catalytic centre of AChE present in the extract.[80] An interesting feature of this study is that these purified membrane fragments are still excitable, in vitro, in the absence of any exogenous source of energy and in the absence of any electrochemical gradient. This suggests that the mechanism of excitation by cholinergic agonists is built into the excitable membrane just as the regulatory properties of allosteric proteins are built into the three-dimensional organization of their polypeptide chains.[77] Changeux has further shown that the AChR-bearing macromolecule can be extracted by deoxycholate in a soluble form and in appreciable amounts from the electric organs of Electrophorus electricus. The macromolecule, a protein distinct from the enzyme acetylcholinesterase, retains in vitro, and in solution, most specific binding properties of the physiological receptor.[81] The receptor protein contains a subunit of approximately 48,000 molecular weight which tightly binds α-bungaratoxin.[82]

In an approach to receptor isolation specifically directed towards extracting a macromolecule presumably intimately bound into the lipoprotein structure of the membrane, De Robertis and his colleagues examined in organic solvents the binding of cholinergic ligands and proteolipids extracted from brain and electric tissue.[83] Receptor properties were localized in the junctional complexes and in the subsynaptic membrane as judged by binding studies with labeled transmitters, synaptic blocking agents and other drugs. When proteolipids extracted from nerve-ending membranes labeled with d-[dimethyl-^{14}C]-tubocurarine were chromatographed on Sephadex LH-20 the receptor-proteolipid was isolated by elution with chloroform and methanol. Similarly, these workers also studied the high affinity for binding ^{14}C-ACh, ^{14}C-hexamethonium, and ^{3}H-TDF by chloroform-methanol extracts of lyophilized electric organs of Torpedo and Electrophorus. After the binding, the lipid extracts were passed through a Sephadex LH-20 column, and the receptor proteolipid eluted with chloroform in a sharp peak which contained most of the bound radioactivity.[83,84] Several physicochemical methods were used to study interaction of drugs and the receptor proteolipid.[83] Notably, atropine sulfate produced changes in light scattering and polarization of fluorescence of the receptor from cerebral cortex, and this effect was blocked by ACh, succinylcholine and hexamethonium.[85]

The concentrations of receptor binding sites calculated by De Robertis and coworkers from their data for electric tissue of eel and Torpedo are much higher than those determined for these tissues by other workers.[47] Moreover, the binding experiments by these authors have been carried out in nonionic, nonpolar solvents, under which circumstances the

cholinergic ligands which normally act reversibly were bound irreversibly. Thus, it has not been possible to determine affinity constants, which makes it difficult to compare their results with those of other laboratories. Recently some evidence has been presented that association of ligands with proteolipids obtained by purification on Sephadex LH-20 may be artifactual.[86]

Acetylcholinesterase and Acetylcholine Receptor: The fact that high concentrations of AChE are present at the neuromuscular junction and that this enzyme has several properties in common with the receptor indicate that they may be part of the same macromolecule, and that their anionic sites could be fully or partially coincident. However, on the basis of autoradiographic studies in the postsynaptic membrane of endplates (mouse diaphragm), Waser demonstrated that cholinergic receptors are different from the active centers of the AChE.[87] Several other pieces of data demonstrating this point are available, and have been summarized by O'Brien et al.[47] and by Belleau.[88] Recent work on receptor isolation has shown it is possible to obtain separation of AChE activity from AChR. Using elution profiles in Triton, Miledi et al.[66] have shown that the receptor and enzyme are clearly separable into two distinct macromolecules. This is consistent with the observation that they are found on two different membrane fragments, and with the fact that the two proteins can be separated by ultracentrifugation.[89]

Nevertheless, the striking similarity in the number of active sites of esterase with the number of toxin-binding or receptor sites observed by various workers requires explanation.[56,63,66,90] The most likely explanation, of course, is that the receptor and the enzyme are linked, perhaps as part of a polymer in the membrane. Belleau further suggests that the anionic regulatory unit of AChE is structurally homologous or analogous to the cholinergic receptor.[91] In several experiments it has been shown that ACh agonists and antagonists must bind also to AChE since they affect the kinetics of hydrolysis of ACh[92] and the methanesulfonylation of AChE.[93] These kinetic effects are explained on the basis of conformation changes initiated in the anionic chain.[91]

The recent crystallization and preparation of pure AChE on a large scale have made it possible to initiate meaningful investigations into the physical and chemical properties of the enzyme.[94] The molecular weight of AChE has been determined by sedimentation equilibrium to be 260,000.[95] The enzyme is split in the presence of guanidine and mercaptoethanol into four subunits, each of equal molecular weight. Examination of the C-terminal amino acid residues indicated that there were two types of polypeptide chains in AChE, one terminating in serine and the other in glycine. Two active sites were found for AChE per 260,000 molecular weight, which is consistent with a proposed dimeric structure of $\alpha_2\beta_2$ type.[96] The presence of a two-fold symmetry in the number of sites is further supported by labeling experiments with TDF and by equilibrium dialysis experiments performed with decamethonium. According to Leuzinger the α and β subunits of AChE fulfill different functions.[94] Either the α-chain of AChE contains the catalytic site while β-chain bears some other

function not yet known, or alternatively, α- and β-chain together form an active center in which α would furnish the esteratic site and β the anionic site. This hypothesis assumes that the β-chain is identical with AChR, a contention supported by the fact that TDF perferably labels the β-chain of AChE[94] and also interacts in vivo with AChR in an inhibitory way.[59] There is also some thought that in an evolutionary sense the β-chain (receptor) may have derived from the α-chain through mutation in the sequence of the amino acids of the esteratic site of the original AChE.[94] Until an X-ray examination of the crystalline enzyme is possible, a sequence analysis of the two separated polypeptides would be of great interest.

Receptor Reconstitution: Prevailing ideas about membrane ultrastructure have been analyzed in an exquisite review by Hendler.[97] Membrane reconstitution is a promising approach to the elucidation of the molecular organization of proteins and lipids in the biomembrane.[98] Recently, interesting results have been obtained with reconstitution of an active transport system. Redwood et al.[99] and Jain et al.[100] studied the binding of membrane ATPase to a bilayer and obtained a system showing the characteristics of the Na^+-K^+ pump of biomembranes. Equally impressive is the demonstration by De Robertis[83,101] that a biophysical response to ACh can be evoked in artificial lipid membranes.

AChE in situ and in solution have markedly different properties. Of special interest in this regard is the intriguing observation of Massoulié and Rieger[102] that at least in the electric eel and in the torpedo fish there is no enzyme in situ which corresponds to the purified enzyme. Studies in which AChE has been incorporated into bilayers having a definite structure similar to natural membranes are therefore of special significance. Leuzinger found that it is possible to observe changes in the permeability of lipid bilayers to ions by incorporating AChE into artificial membranes.[103] These results have been confirmed and extended by Jain et al.[104] who found that the dramatic increase in membrane conductance elicited by addition of small concentrations of ACh or carbamylcholine to a black lipid membrane incorporating electroplax AChE is prevented by addition of neostigmine or atropine to the system. The magnitude of the conductance increase of the AChE-treated membrane is proportional to the fourth power of the carbamylcholine concentration suggesting that four AChE subunits or molecules aggregate to form a potential ion-conducting channel. A separate cholinergic receptor independent of the AChE $\alpha_2\beta_2$ complex appears unnecessary on the basis of these experiments.[103]

Conclusion: Discernible progress is being made towards isolation and purification of receptor macromolecules. Biochemical characterization of the cholinergic receptor would be an important landmark in the development of molecular pharmacology, and would eventually pave the way to a clearer understanding of the pharmacological problems of drug-receptor interaction and a fuller explanation of the molecular basis of drug action.

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Chapter 28 - Immediate Hypersensitivity: II. Drugs in Clinical Use

Elliott Middleton, Jr., & Ronald G. Coffey, CARIH, Denver, Colo.

Introduction - The accumulation of knowledge in the field of immunology has taken place at a dazzling rate in recent years. Work in the field of immediate hypersensitivity reactions has been especially rewarding and the fundamentals were well presented by Bach.[1] This article will review the currently accepted pharmacologic approaches to management of the clinical immediate hypersensitivity states, some hopeful glimmers for future drugs and also will attempt to relate the effect of these agents to their biochemical mechanisms of action.

Diseases of Immediate Hypersensitivity - By definition, the diseases of immediate hypersensitivity are reagin-mediated, i.e., the pathophysiological changes characteristic of the condition are initiated by the interaction of a specific antigen with homologous immunoglobulin E (IgE) antibody bound to tissue mast cells or circulating basophils. The classical diseases of immediate hypersensitivity are seasonal and perennial allergic rhinitis and asthma, anaphylaxis, certain cases of urticaria and angioedema, occasional cases of atopic dermatitis (eczema), certain instances of food and drug reactions and bee sting hypersensitivity. Patients with reagin-mediated disorders usually show positive skin or mucosal tests to appropriate antigens (at low concentrations), often have elevated levels of serum IgE and incubation of their peripheral leukocytes results in the release of histamine from basophils. It is important to understand, however, that these conditions may occur in individuals in whom it is impossible to demonstrate an underlying immunologic reaction mechanism. In other words, demonstrable allergy is a sufficient but not an exclusive cause of the syndromes, even though the pathology of the non-allergic state may be indistinguishable from the allergic. This points to the probability of there being other fundamental defects in cell biochemistry that are of pathogenetic importance whether allergy coexists or not. In short, the allergic reaction can be one of several triggers that precipitates the clinical condition. Nevertheless as of this writing, pharmacologic management differs little between the allergic and non-allergic groups.

Pathophysiologic Events - Whether or not allergy is a specific precipitant, there are a number of pathophysiologic events one or more of which contributes to the development of the typical clinical picture. These tissue events include increase in vascular permeability, smooth muscle contraction, mucous gland hypersecretion, leukotaxis and especially eosinophilotaxis, and irritation of sensory nerve endings. Each of these tissue changes, in the case of immunologically mediated tissue injury, can be accounted for by one or more of the chemical mediators of immediate-type allergic inflammation: histamine, slow reacting substance of anaphylaxis (SRS-A), eosinophil chemotactic factor of anaphylaxis (ECF-A) and possibly the kinins, prostaglandins and others yet to be discovered.[2,3] How these tissue changes come about in the absence of an immunologic reaction remains to be determined but in part it may have something to do with the final expression

of certain cell enzyme activities reflecting altered adrenergic and cholinergic functions, as will be described.

Immunologic chemical mediator release by the involved cells is a non-cytotoxic process requiring a proesterase, an intact glycolytic system and calcium and can be modified by drugs and hormones which influence levels of cyclic nucleotides.[3-11] In the case of histamine, release from the cell appears to be a secretory process probably involving microtubular structures.[12,13] Neither SRS-A nor ECF-A exist in (lung) tissue in a preformed state (or if so, only in minute quantities). Therefore, the immunologic reaction at the cell membrane must activate a mechanism that leads to their formation and subsequent release. Once released the chemical mediators must traverse intercellular material to interact with specific receptors on effector cells such as smooth muscle, mucous glands, vascular cells, nerve endings and perhaps others, in which they produce a particular change such as contraction, secretion, permeability changes, and sensory nerve stimulation.

Relationship of Cyclic AMP and Cyclic GMP to Chemical Mediator Release and Smooth Muscle Function - Before discussing various types of drugs used in clinical management, it is necessary to review briefly the cyclic nucleotides, cyclic 3',5'-adenosine monophosphate (cyclic AMP) and cyclic 3',5'-guanosine monophosphate (cyclic GMP), and their relationship as presently understood to chemical mediator release and smooth muscle function. Their production and intracellular concentrations are controlled by certain key enzyme systems the activities of which are modified by many substances including some of the drugs to be discussed.

Cyclic AMP is formed from ATP and the reaction is catalyzed by the cell membrane enzyme adenylate cyclase, the activitiy of which is known to be stimulated by many hormones[14] including catecholamines, certain prostaglandins and histamine. Cyclic GMP is produced from GTP by action of the enzyme guanylate cyclase[15,16] Several substances such as acetylcholine, bradykinin, prostaglandin $F_{2\alpha}$ and insulin are known to increase cyclic GMP formation.[17] Cyclic AMP and cyclic GMP are hydrolysed in the cell by cyclic nucleotide phosphodiesterases which may have varying affinities for the respective cyclic nucleotides.[18,19] The intracellular concentration of these substances is, therefore, a balance between the hormonal driving forces causing their formation and the rate of hydrolysis by phosphodiesterases within the cell.

Since catecholamines can stimulate accumulation of cyclic AMP and acetylcholine can stimulate accumulation of cyclic GMP, it is possible to consider that at least in certain cells the concentrations of these cyclic nucleotides is a biochemical reflection of the activity (and balance) of the sympathetic (adrenergic) and parasympathetic (cholinergic) divisions of the autonomic nervous system. The possible importance of such a relationship in certain disorders, e.g., asthma is evident.

Evidence to date indicates that cyclic AMP and cyclic GMP have a sort of reciprocal or Yin Yang relationship in cell function.[17] Such a relation-

ship appears to hold for the state of smooth muscle contraction[17] and possibly for chemical mediator release,[11] i.e., manipulations that increase intracellular cyclic AMP concentrations lead to smooth muscle relaxation and decreased release of chemical mediators, whereas increases in cyclic GMP leads to the opposite effects.

Various therapeutic measures (and clinically aggravating factors) may relate closely to these critically important enzyme systems and cyclic nucleotides.

Sympathomimetic Amines - Pharmacological dissection of adrenergic receptors has led to a subdivision into alpha and beta-1 and beta-2 classes that are stimulated to different degrees by various sympathomimetic amines.[20,21] Beta-2 receptors are present in tracheobronchial smooth muscle and when stimulated cause relaxation. Sympathomimetic amines have long been useful in management of various disorders, especially asthma, anaphylaxis, rhinitis, urticaria and angioedema and bee sting reactions. Classic examples are epinephrine, ephedrine and isoproterenol. Their salutory effects reside mainly in their ability to stimulate beta adrenergic receptors but they may also have alpha receptor stimulating activity and ephedrine also has indirect actions, i.e., it causes release of catecholamines. The beta agonistic catecholamines stimulate intracellular formation of cyclic AMP and cause smooth muscle relaxation[22] and inhibition of mediator release especially in the presence of a phosphodiesterase inhibitor such as theophylline.[11] Alpha adrenergic agents, on the other hand, may actually enhance mediator release while lowering cyclic AMP levels.[11]

The usefulness of the older sympathomimetics is limited by certain undesirable side effects, especially cardiac, namely increased force and rate of contraction, mediated by stimulating of beta-1 receptors. Certain more recent derivatives, however, are freer of the beta-1 stimulating properties and act mainly on beta-2 receptors, which subserve smooth muscle relaxation, necessary for bronchodilation in asthma. Since asthma is one of the most common and serious disorders under discussion, the availability of a selective nontoxic bronchodilating agent would be welcomed. The combined use of sympathomimetic amines and a specific beta-1 receptor blocking compound such as practolol has been suggested[23] but is not without risk of aggravating asthma.[24]

Bronchodilator drugs, especially given by aerosol, act on mucosal blood vessels and smooth muscle. In certain cases mucosal edema may be a prominent feature and the alpha receptor stimulating vasoconstrictor sympathomimetics may be helpful. Relief of bronchial smooth muscle spasm is best achieved with beta-2 stimulating drugs. The most selective of these are salbutamol, terbutaline, and isoetharine which probably act as the best (most selective) activators of adenylate cyclase.[25] The saligenin (salbutamol) and resorcinol (terbutaline) derivatives are not metabolized like isoproterenol to compounds with beta adrenergic blocking properties (3-methoxy-isoproterenol). The duration of action of salbutamol is considerably longer than isoproterenol and it can also be given by mouth without causing significant cardiovascular side effects. Of course, the effective-

ness of any beta-2 stimulant depends ultimately on the availability in tissue of active receptors. In many patients with asthma, the beta receptors are not functionally normal, as borne out by studies of the theory of beta adrenergic blockade of asthma.[26-31]

Phosphodiesterase (Pde) Inhibitors - Pde inhibitors act by preventing to some degree the hydrolysis of intracellular cyclic AMP and also cyclic GMP. The methyl-xanthines are the best known class of compounds with this property and theophylline is the best known example. Asthma is frequently treated with theophylline compounds.

A very practical point about the clinical pharmacology of theophylline is that different patients exhibit different rates of metabolism so individualization of dosage becomes important.[32]

Several forms of cyclic nucleotide Pde are known to exist with different enzymatic properties, affecting hydrolysis of both cyclic AMP and cyclic GMP.[18,19] Indeed, there is evidence that the concentration of cyclic GMP in the cell has a controlling influence on cyclic AMP levels.[18] Much work is underway to synthesize Pde inhibitors with selective action on specific enzymes and with specific tissue affinities. Chemical modification of the xanthine nucleus has provided Pde inhibitors of various potencies. Several imidazopyrazines have been found to have inhibitory activity against Pde in certain tissues including rat lung.[33] Two of these compounds had bronchodilator activity. A triazole compound (3-acetamido-6-methyl-8-n-propyl-s-triazole, I.C.I. 58, 301) protected guinea pigs against histamine aerosol and at low concentrations decreased the spasm of isolated guinea pig lungs perfused with histamine, acetylcholine, serotonin or bradykinin.[34] It was reported to have no cardiovascular or central nervous system effects. In man the drug did not have bronchodilator activity but it may find use as a preventative of bronchoconstriction.[35] Also, certain imidazolidinone derivatives are potent Pde inhibitors with special activity in smooth muscle (beta-2) preparations.[36] Bronchodilator activity and guinea pig tracheal strip relaxing activity were reported. The ultimate clinical utility of any of these compounds remains to be determined.

Corticosteroids - Much of the literature on metabolism and clinical use of corticosteroids in asthma pertinent to this review has been summarized.[37] Among the many corticosteroids studied in the management of various immunologically mediated diseases several have outstanding anti-inflammatory, anti-allergic activities with relatively slight tendency to produce adverse side effects. These are hydrocortisone (cortisol), prednisone, 6-methylprednisolone, and prednisolone, all of which are relatively short acting and therefore possess less adrenal suppressive effects. These hormones are frequently used in the management of the more severe stages of immediate hypersensitivity diseases, can be life-saving in status asthmaticus and permit a more or less normal existence for patients with intractable asthma. Most often they are administered by oral, intravenous or topical (allergic dermatoses) routes. When given orally, it is now accepted that alternate day therapy given in the morning is both therapeutically effective and less likely to cause undesirable side effects.[37]

Recently, a new steroid, beclomethasone, has been introduced for aerosol administration in the treatment of asthma.[38,39,40] As an alternative to oral therapy at proper dosage it is reported to be very helpful and free of adrenal suppressive properties by systemic absorption but this may occur if excessive quantities are inhaled.[41]

The mechanism of action of corticosteroids in modifying allergic inflammation isn't well understood. However, recently it has been shown that in patients with asthma, administration of corticosteroids restores responsiveness of leukocyte adenylate cyclase to isoproterenol stimulation,[29] whereas untreated patients usually show absent or depressed leukocyte adenylate cyclase stimulation with isoproterenol.[29,30] Also leukocyte and platelet ATPase activities are generally elevated in asthma and patients receiving corticosteroids show a reversal of the abnormality.[42] Since ATPases are known to be associated with certain contractile, secretory and transport processes, the possible importance of this finding is evident. Also, in high concentrations hydrocortisone has been shown to inhibit Pde as well as ATPase[42] in broken cell preparations. Finally, hydrocortisone has a direct stimulating effect on leukocyte adenylate cyclase activity.[29] It also is claimed that corticosteroids may directly relax smooth muscle and inhibit endogenous histamine formation.[43] Of great interest is the observation[17] that cortisol treatment significantly reduced rat lung cyclic GMP levels. If bronchoconstriction is indeed mediated by cyclic GMP as suggested by experiments with other smooth muscle preparations,[17,22] this finding may represent the most significant advance in our understanding of the mechanism of glucocorticosteroid action.

Prostaglandins - The prostaglandins may play a role in allergic inflammation[44] and yet certain prostaglandins may have therapeutic potentialities.[45] The prostaglandins have many pharmacological actions especially affecting smooth muscle systems and they are known to affect adenylate cyclase activity.[46] Human lung contains prostaglandins of both the E and F series, E_2 and $F_{2\alpha}$ being most abundant.[47,48] Prostaglandin E_2 and $F_{2\alpha}$ are released from anaphylactically sensitized guinea pig lung in vitro on challenge with antigen.[49] Prostaglandin $F_{2\alpha}$ contracts tracheobronchial smooth muscle whereas PGE_1 and PGE_2 relax.[50]

Human experimentation has disclosed that asthmatics are exquisitely sensitive to the bronchoconstrictor effects of $PGF_{2\alpha}$ administered by aerosol.[51] On the other hand, PGE_1 and PGE_2 have been reported to be effective bronchodilators in some patients with asthma. In animals PGE_2 and isoproterenol were of approximately equal potency but in asthmatics PGE_2 was about one-tenth as potent (on a weight basis) and its peak effect was delayed by a few minutes.[45] Upper airway irritation cough and expectoration may follow PGE_1 or PGE_2 inhalation (more with the free acid than the salt) and occasionally frank aggravation of asthma may occur which can be relieved by isoproterenol.[45]

The in vitro bronchoconstrictor effect of $PGF_{2\alpha}$ (which might be mediated by cyclic GMP[17]) can be inhibited by analgesic anti-inflammatory drugs.[52] The bronchoconstrictor effect of $PGF_{2\alpha}$ following inhalation in

normal subjects could be completely reversed by isoproterenol but only partially reversed by PGE_2.[51,53] Prior inhalation of atropine or disodium cromoglycate did not affect $PGF_{2\alpha}$ bronchoconstriction.[45] $PGF_{2\alpha}$ also induces coughing and a feeling of substernal irritation.

The synthesis of prostaglandins is inhibited by analgesic anti-inflammatory compounds like aspirin, indomethacin and the fenamate drugs (flufenamic and mefanamic acids).[54] While inhibiting synthesis of prostaglandins the analgesics also inhibit the smooth muscle stimulating effects of $PGF_{2\alpha}$ but not the effects of PGE_2 (aspirin, fenamates, and phenylbutazone).[52]

Human bronchial smooth muscle contraction <u>in vitro</u> by $PGF_{2\alpha}$ is also inhibited by polyphloretin phosphate,[55] a high molecular weight polyanionic polyester of phosphoric acid and phloretin. <u>In vivo</u>, in animal experiments, polyphloretin phosphate inhibited $PGF_{2\alpha}$ induced bronchoconstriction but not the relaxant effect of PGE_2.[45] No detailed studies in man have been reported.

Immunologic release of histamine by human leukocytes can be inhibited by prostaglandins in the following descending order of potency: $E_1 = E_2 > A_1 = A_2 > B_1 > F_{1\alpha} = F_{2\alpha} > 0$.[56] The inhibitory capacity coincided closely with the ability of a particular prostaglandin to increase the accumulation of cyclic AMP in leukocytes. A similar effect of PGE_1 and $PGF_{2\alpha}$ on immunologic release of histamine in human lung fragments has been reported[57] and the inhibitory action could be related to increased cyclic AMP levels.

These considerations suggest that prostaglandins may be involved in the control of bronchial smooth muscle tone and thus in the pathogenesis of asthma, possibly through alteration of a normal balanced relationship between $PGF_{2\alpha}$ and PGE_2. This remains to be documented, however. The bronchodilator activity of PGE_2 and E_1 suggests clinical utility but the serious disadvantage of upper airway irritation or induction of bronchoconstriction will have to be overcome. Also, the duration of action is only about one hour, considerably less than the beta-2 agonists. Inhibition of prostaglandin synthesis in the lung (especially $PGF_{2\alpha}$) is a possible approach, i.e., through use of such analgesic anti-inflammatory drugs as mentioned above. Indeed, occasional patients seem to be benefitted by certain of these drugs, but some asthmatics are unquestionably worsened by them and their experimental use in asthma should be carefully monitored.[58,59]

<u>Alpha Adrenergic Antagonists</u> - Several reports suggest that alpha adrenergic blockers such as phentolamine,[60] thymoxamine[61] and dibenamine[62] may be useful in the management of asthma. There is ample evidence that mammalian (including human) tracheobronchial smooth muscle possesses alpha adrenergic receptors[63] that can be stimulated by alpha adrenergic agonists to produce contraction. Relative to cholinergic bronchoconstriction alpha adrenergic effects are probably relatively minor unless there is a significantly greater population or hyper-reactivity of alpha receptors in smooth muscle in asthma. Alpha adrenergic blocking agents (phentolamine and phenoxybenzamine[64] and thymoxamine[65,66]) inhibit the production of airway narrowing caused by histamine. Thymoxamine does not have significant anti-

histaminic properties. A possible mechanism of action of alpha antagonists has been worked out in studies of asthmatic leukocytes.[67] Leukocyte adenylate cyclase could be directly stimulated by phentolamine and this effect could be blocked by propranolol suggesting that phentolamine was acting as a beta adrenergic agonist. Also, isoproterenol responsiveness of asthmatic leukocyte adenylate cyclase was restored by phentolamine.

Disodium Cromoglycate (DSC) - DSC now enjoys an established reputation as an effective adjunct in the management of asthma,[68,69,70] as well as possibly reducing Type III immunologic lung injury.[68] It is administered as an aerosol and is non-toxic. Its major advantage is that it frequently allows a reduction in steroid requirements. Its mechanism of action is unknown but the principle effect is the inhibition of mediator release in sensitized tissues occurring at a step after antigen-antibody reaction and before mediator release. The inhibition of bronchoconstriction in allergic asthmatics on inhalation of allergens can be prevented by DSC as can exercise-induced asthma.[71] DSC does not interfere with the union of antigen and antibody, does not antagonize the effects of histamine or SRS-A and is not a direct bronchodilator. It is most effective when administered shortly before antigen. Clinically, its effectiveness is not limited to reagin-mediated asthma; it is also helpful in patients with intrinsic asthma, suggesting that it may affect some fundamental cell processes related to the production of bronchoconstriction, e.g., stabilization of mast cell membranes.[72] By the nasal route, it may afford relief in allergic rhinitis[73-76] and topically in vernal keratoconjunctivitis.[77]

Inhibition by DSC of in vitro mediator release from sensitized human lung exhibits a peculiar dose response relationship.[3] Significant inhibition is noted at 0.1 to 10 μg/ml but at higher concentrations the inhibitory effect vanishes. Thus, it is conceivable that clinical effectiveness could be lost with excessive dosage. In rat passive cutaneous anaphylaxis experiments it was found, in the presence of DSC, tissue mast cells may be desensitized to one antigen without altering their capacity to react to a second antigen with mediator release. This observation raises the interesting therapeutic possibility of specific desensitization to antigens administered with DSC.

Diethylcarbamazine (DEC) - This antifilarial drug appeared to have a beneficial effect on asthma while it was being given for concurrent infestation.[78] Subsequently, it was shown to inhibit immunologically mediated release of SRS-A in the rat,[79] and both histamine and SRS-A from monkey and human lung.[80] A synergistic effect of DEC with beta adrenergic agents but not with methyxanthines was noted and no obvious relationship to the adenylate cyclase-cyclic AMP system has been shown. A related compound, pipecolamide, was even more active than DEC.[81] Like DSC, DEC appears to act after union of antigen and antibody and before chemical mediator release.[82]

Clinical studies are limited in number and to date no major use for DEC has emerged in asthma therapy.[83,84]

Atropine - Asthma has been considered possibly to be a disorder of autonomic

imbalance with parasympathetic (cholinergic) predominance, and that the characteristic hyperirritability of the airways may be attributable to this. Recent evidence supports this old contention.[85] Stimulation of the subepithelial irritant receptors of the tracheobronchial tree by dusts, SO_2, cold air or antigen in sensitized subjects (dogs, humans) initiated a vagal reflex which induced bronchoconstriction.[85,86,87] The reflex increase in airway resistance could be abolished by interrupting the vagal afferent or efferent limbs of the reflex or by the administration of atropine sulfate intravenously or by aerosol. Clinically, the major disadvantage of atropine is its drying effect which could favor mucous plug formation. There may be, however, certain patients who would respond well to anticholinergic drugs and there is renewed interest in clinical research in this area. Since anaphylaxis is associated with increased vagal afferent and efferent discharges[85] and since cholinergic (muscarinic) drugs enhance mediator release,[11] it is possible that atropine may inhibit mediator release as well as inhibit cholinergically induced smooth muscle contraction. Since cholinergic stimulation brings about increased cyclic GMP accumulation in certain tissues,[17] it may be that increased cyclic GMP levels favor increased mediator release and smooth muscle contraction and that atropine inhibits this sequence.

Cyclic AMP Analogs - If increased levels of cyclic AMP tend to diminish the deleterious effects of immediate hypersensitivity reactions, then it is logical to consider cyclic AMP itself or some of its analogs to be useful therapeutic agents. A number of analogs have been synthesized and some have smooth muscle relaxing effects[36] and inhibit chemical mediator release from lung tissue in vitro.[11] Clinical usefulness of any analogs remains to be determined.

Antihistamines - These drugs have been used for many years in the management of many allergic disorders most notably allergic rhinitis and urticaria. Antihistamines may either worsen or improve asthma.[88] In general, they act by inhibiting the action of histamine on target cell receptors. However, some antihistamines at concentrations of 10^{-6} and 10^{-5}M are said to be nontoxic reversible inhibitors of antigenic histamine release.[10] Further study of this action is certainly warranted.

Miscellaneous - Chlorphenesin, a muscle relaxant carbamic acid derivative, inhibits immunologic histamine release in human leukocytes[89] and both histamine and SRS-A release from monkey lung.[90] Trimetoquinol, a unique tetrahydroisoquinoline derivative, has beta agonistic and papavarine-like qualities[91] and is a good clinical bronchodilator.[92] An alcoholic extract of tylophora indica is said to be an effective long lasting bronchodilator.[93] Its mechanism of action is unknown.

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Chapter 29. Delayed Hypersensitivity: Its Mediation Through Products of Activated Lymphocytes

Ross E. Rocklin, M.D., Allergy-Immunology Service, Walter Reed General Hospital, Washington, D. C. 20012

The existance of delayed hypersensitivity or cell-mediated immunity has been known for some time but its importance in health and disease has only recently been appreciated. Some of the functions ascribed to cell-mediated immunity include: resistance to infection by certain bacteria, fungae, viruses and intracellular parasites (1), immunologic responses to neoplastic cells(2), rejection of homografts(3) and some autoimmune diseases(4). It has also been established that cell-mediated reactions involve both immunologically specific and non-specific components. The reaction of antigen with a few specifically sensitized lymphocytes results in the recruitment of a large number of non-sensitive macrophages(5). Recognition of the central role played by lymphocytes and macrophages in this reaction has stimulated intensive study into its mechanism. It has been proposed, on the basis of much in vivo and in vitro experimental work, that the interaction of these cells may result from the production of soluble lymphocyte factors(Reviewed in 6,7). Moreover, the production of these mediators may serve as a means of communication between sensitive lymphocytes and macrophages and perhaps other cells(polymorphonuclear leukocytes) involved the cell-mediated reaction. Some of the properties of these substances and their role in cell-mediated immunity will be discussed as well as their clinical significance in human disease.

Definition of Delayed Hypersensitivity or Cell-Mediated Immunity- A Classic example is the Tuberculin PPD skin reaction. When tuberculin is injected intradermally into an appropriately sensitized individual, there ensues an inflammatory reaction characterized by erythema and induration which reaches its peak within 24-48 hrs and gradually subsides. It differs from immediate hypersensitivity or arthus reactions because the latter phenomena begin minutes to a few hours, respectively, after injection of antigen and are mediated by antibodies. Cell-mediated reactions, on the other hand, are mediated by immunocompetent lymphocytes or cells having the potential to engage in an immunologic reaction.

Lymphocyte Mediators- When lymphocytes are activated by non-specific mitogenic(eg. phytohemagglutinin, Concanavalin A) or specific antigenic (eg. Tuberculin PPD, Monilia, Streptokinase-streptodornase) stimuli in vitro a number of soluble substances are elaborated which have a variety of biological activities(8). Some of these are listed in Table 1. Because these mediators are produced in such minute quantities and have never been isolated in pure form, several problems remain unanswered; 1) whether these biologic activities are produced by the same molecule assayed in different in vitro systems or 2) whether these are all chemically distinct molecules and 3) how many of these substances are relevant to the in vivo state of cell-mediated immunity. Evidence will be summarized which suggets that at least some of these biological activities can be separated

by physico-chemical means. Moreover, their function in in vivo cell-mediated immunity may be to serve as biologic amplifiers, attracting and recruiting other inflammatory cells in the microenvironment to participate in the reaction.

Table 1. Mediators Elaborated by Activated Lymphocytes

1) Migration Inhibitory Factor (MIF)
2) Macrophage Aggregating Factor
3) Macrophage Activating Factor
4) Chemotactic Factors for: Macrophages, Neutrophils, Eosinophils and Lymphocytes
5) Cytotoxic Factor or Lymphotoxin
6) Growth Inhibitory Factors: Clonal Inhibitory Factor
   Proliferation Inhibitory FActor
6) Skin Reactive Factors
7) Blastogenic or Mitogenic Factor for Lymphocytes
8) Interferon
9) Transfer Factor

Migration Inhibitory Factor(MIF)- Sensitized lymphocytes, when stimulated in vitro by specific antigen, elaborate into the culture medium a soluble material that inhibits the migration of normal peritoneal exudate cells (macrophages) from capillary tubes(9-11). MIF was produced only in the presence of specific antigen and was not found in the cell-free supernatants of lymphocytes cultured in the absence of antigen or in the presence of unrelated antigen. It was detected within the first 24 hours of culture and for as long as 4 days(12). The inhibitory activity was found to be non-dialysable and heat stable at 56°C for 30 minutes(13). MIF activity is not diminished when the material is treated with RNAse and DNAse(14) but is inactivated by enzymatic digestion with Trypsin(15) and chymotrypsin(16). Furthermore, puromycin (an inhibitor of protein synthesis) prevents MIF production by lymphocytes and also affects macrophages so that they no longer respond to MIF. Actinomycin D(an inhibitor of DNA dependent RNA synthesis) also prevents lymphocytes from elaborating MIF(17). Immunosuppressive agents such as cortisone and prednisone do not prevent MIF production in vitro in non-toxic doses although they are effective in obliterating the delayed hypersensitivity skin test(7). Their effect may be on the macrophage response to MIF(18).

Attempts to estimate the size of MIF by sucrose density centrifugation and gel filtration on Sephadex(G-100) indicate that guinea pig MIF has a MW 35-55,000(19) and that human MIF is slightly smaller (MW 25,000) (20). Results employing electrophoresis on acrylamide gels demonstrated that guinea pig MIF migrated anodally to albumin(more acidic)(19), and that incubation with neuraminidase(cleaves terminal sialic acid residues) destroys its activity, suggesting that sialic acid moieties may be necessary for its biologic activity(16). Isopycnic centrifugation (determines buoyant density) verified the glycoprotein nature of guinea pig MIF(16). Human MIF appears to be less acidic(migrates with albumin on gel electrophoresis), is not inactivated by neuraminidase, and has the buoyant

density of a protein(20)

A factor(macrophage aggregating factor) has also been described which causes the agglutination of macrophages in test tubes and is probably similar if not identical to MIF(21).

Macrophage Activating Factor - Studies in mice have shown that macrophages isolated from animals surviving an infection by intra-cellular organisms such as L. Monocytogenes appear "activated" when observed in vitro(22). These cells are larger, have higher rates of phagocytosis and are more bactericidal for a variety of organisms than macrophages from non-immune animals. The question raised by these findings is whether this activated state can be simulated in vitro by mediators such as MIF and indeed preliminary studies indicate a possible role for these substances. Several investigators have shown that culture supernatants obtained from antigen-stimulated lymphocytes cause macrophages to become more adherent to their culture vessel(23) and more bactericidal(24). Furthermore, Nathan et al (25) have shown that macrophages from normal guinea pigs become enlarged, exhibit increased ruffed membrane activity, motility, glucose oxidation through the Hexose Monophosphate Shunt Pathway, and rates of phagocytosis following exposure in vitro to partially purified MIF. Macrophages activated with supernatants containing MIF activity were also protected from extensive infection by Listeria monocytogenes(26). The factor which activates macrophages and that which inhibits macrophage migration (MIF) are at present inseparable by physico-chemical means. Macrophage activating factor, like MIF, has a molecular weight of approximately 35-55,000, behaves as an acidic glycoprotein and loses its activity by incubation with neuraminidase(27). Human blood monocytes are also activated by human lymphocyte mediators(28). It is of interest that activation is observed after 3 days but migration inhibition of these cells can be detected within 24 hours. If the two effects on macrophages were mediated by the same substance, then the initial event would be a slowing of migration followed by an increased rate of migration after several days.

Chemotactic Factor - Although chemotactic activities have been described for most inflammatory cells, only the macrophage factor has been characterized in some detail. This material, produced by antigen-stimulated lymphocytes, induces rabbit, guinea pig or human monocyte-macrophages to migrate thru a millipore filter toward the chemotactic gradient(29). Like MIF, it is non-dialysable, is heat stable at 56°C for 30 minutes and has a similar MW(35-55,000)(30). Chemotactic factor can, however, be separated from MIF by disc electrophoresis(30). When Sephadex fractions containing chemotactic factor were further fractionated by acrylamide gel electrophoresis, mononuclear chemotactic activity was regularly found in the same gel fraction with albumin; by contrast, MIF migrated anodally to albumin. In addition, chemotactic factor has a buoyant density similar to that of protein(albumin) and does not lose its activity when incubated with neuraminidase(31).

Cytotoxic Factor or Lymphotoxin - Cytotoxic factors have been produced by sensitized lymphocytes in response to specific antigen(32) and with

mitogens(33). This material is assayed in vitro by its lytic effect on susceptible target cells such as mouse fibroblasts. Guinea pig lymphotoxin is heat labile at 56°C for 30 minutes and thus differs in this respect from MIF and chemotactic factor(32). It is also non-dialysable, has a MW of 35-55,000, migrates with albumin gel electrophoresis, has a buoyant density similar to that of protein and is resistant to treatment with neuraminidase(31). Human lymphotoxin is larger (MW 80-90,000), resistant to DNAse and RNAse and trypsin(34). Its production by lymphocytes is prevented by drugs such as puromycin, cyclohexamide, 2.4-DNP and cortisone(35). Furthermore, Cytochalasin B (a drug which affects microfilament and other cell functions) has also been shown to interfere with the production of lymphotoxin by sensitive lymphocytes (35a).

Growth Inhibitory Factors - Clonal inhibitory factor is a substance produced by antigen stimulated human lymphocytes which prevents the cloning of HeLa cells in culture(36). The material is non-dialysable and heat labile(56°C for 30 minutes). This inhibitory effect on the growth cycle appears to be reversible and differs from human lymphotoxin because it does not kill the target cell. Proliferation inhibitory factor is an activity detected in cell-free supernatants obtained from mitogen stimulated lymphocytes(37). This material prevents the proliferative response of HeLa cells and other human tissue culture lines in vitro. Proliferation is measured by incorporation of 3H-thymidine into cell DNA. The factor is stable at 85°C for 30 minutes, non-dialysable, non-sedimentable at 90,000g and is destroyed by trypsin. These factors and lymphotoxin may serve to kill or suppress the growth rate of rapidly proliferating cells (e.g. neoplasms).

Skin Reactive Factors - The appearance of an accelerated delayed-type hypersensitivity reaction resulting from the injection of Sephadex fractions containing MIF activity into the skin of normal guinea pigs gave the first indication that the lymphocyte derived mediators may have some in vivo importance(38). The skin reactive factor could be induced by stimulating lymphocytes with specific antigen(39,40) or with mitogens(41,42). The reaction was characterized by the development of erythema and induration at 3-5 hrs, reaching a peak by 8-12 hours and disappearing by 30 hours. Histologically the lesion simulated a delayed hypersensitivity reaction in that the infiltrate was comprised primarily of mononuclear cells(38). This activity could be partially destroyed by trypsin and papain but was resistant to DNAse and RNAse(43). Furthermore, drugs such as puromycin, chlorphensin and actinomycin prevented its production(41). Cortisone, however, had no effect on its production in vitro although it could prevent its expression in vivo(43). Since the preparation used for in vivo experiments contain MIF, chemotactic factor and lymphotoxin, it is not clear at present whether one or a combination of these are responsible for the skin reaction.

Blastogenic or Mitogenic Factor - Supernatants from mixed human leukocyte cultures(44) and antigen stimulated lymphocytes(45,46) have been shown to contain materials which activate non-sensitive lymphocytes so that they increase their DNA synthesis and undergo blast transformation. The factor

is non-dialysable, heat labile at 56°C for 30 minutes and not sedimentable at 100,000 g. The existance of a mitogenic factor which can activate or recruit non-sensitive lymphocytes to participate in an on-going reaction could provide a mechanism for expanding a cellular reaction and enlarging the population of cells that are producing mediators.

Interferon - Lymphocytes have been shown to produce interferon when stimulated by various viral interferon inducers(47) and by specific antigen(48). The material is stable at pH 4-10 at 4°C for 24 hours, non-sedimentable at 100,000 g. for 2 hours, resistant to DNAse and RNAse, but destroyed by trypsin. Its effect was specific for cells of human origin and did not protect mouse fibroblasts, chicken embryo fibroblasts or rabbit kidney cells against the viruses tested. The production of interferon during or following a specific immunologic reaction may be of great importance to the host. Since patients with depressed cellular immunity are plagued with viral infections it will be of interest to learn whether the antigen-induced interferon produced by lymphocytes could play a significant role in the development of immunity to these organisms.

Transfer Factor - Studies by H.S. Lawrence have demonstrated that delayed hypersensitivity may be transfered in man by means of white blood cell extracts(49). If one prepares an extract from the leukocytes of a human donor with a positive skin test to Tuberculin PPD(or other antigens) and injects it into a recipient who lacks PPD sensitivity, the receipient will develop a positive PPD skin test (or other positive skin test of the donor) in 1-2 days. This dialysable material appears to sensitize lymphocytes so that they can respond to specific antigen if subsequently stimulated by that antigen(50). Following stimulation, these sensitive cells can proliferate and produce mediators such as MIF(49). The mechanism by which transfer factor permits lymphocytes to respond to specific antigen is not known at present. In addition, little is known concerning the nature of the active moiety. The material is known to be resistant to digestion by DNAse, RNAse and trypsin and its MW is less than 10,000.

Clinically, transfer factor appears to show promise as a means of immunologically reconstituting patients with depressed cellular immunity. Thus it has been used to treat patients with chronic or recurrent infections such as chronic mucocutaneous candidiasis(51), Wiscott-Aldrich syndrome(52), leprosy(53), tuberculosis(54), and coccidiomycosis(55). In many of these cases antibiotics alone were not effective in erradicating the infections or if effective in preventing recurrences. The administration of transfer factor in conjunction with appropriate antibiotics would appear to be a useful approach to therapy in these patients.

Clinical Significance of Mediators - Studies have shown that the production of soluble mediators by lymphocytes from normal subjects generally correlates with the in vivo state of cell-mediated immunity of the donor. These substances are not produced by antigens which fail to elicit a positive delayed skin test (56,57). It is of interest then that lymphocytes from patients with depressed cell-mediated immunity and cutaneous anergy (negative delayed skin tests to multiple antigens) do not produce these

mediators in response to antigenic stimulation. For example, lymphocytes from some anergic patients with the DiGeorge syndrome or thymic aplasia (58), sarcoidosis(59), chronic mucocutaneous candidiasis(60), Wiscott-Aldrich syndrome(52), Hodgkins disease(61), and Rheumatoid arthritis(62) do not produce MIF. In some cases, however, the proliferative response to antigens and mitogen may be normal although MIF production is depressed (63). The cutaneous anergy in these patients may be due to a lack of mediator production by their lymphocytes. Of interest is the finding in other anergic patients that MIF production and the proliferative response is normal indicating intact lymphocyte function(61). Perhaps abnormalities in macrophage function, the inflammatory response per se or in the skin might explain the anergy observed in these latter patients.

The MIF assay has also been of use in the detection of sensitized lymphocytes in patients with certain diseases whose pathogenesis may involve an immune mechanism. For example, it can be shown that lymphocytes from certain patients with glomerulonephritis produce MIF in response to glomerular basement membrane antigens(64,65). Furthermore, lymphocytes from some of these patients also react to group A streptococci, antigens known to cross-react immunologically with human glomeruli(66). Although these studies suggest that the lymphocyte may play a significant role in the pathogenesis of certain glomerular lesions, they do not permit one to distinguish whether this involvement is primary to the initiation of damage or secondary and causing progression of the disease. Similar findings of MIF production by sensitized lymphocytes in response to tissue antigens have been demonstrated in patients with thyroiditis(67), pernicious amenia (68), Multiple Sclerosis(69) and the Guillain-Barre syndrome(69).

Relation of Lymphocyte Mediators to In Vivo Delayed Hypersensitivity

That products of activated lymphocytes may play a role in the expression of delayed hypersensitivity was shown by studies involving skin reactive factor described earlier. This material induces mononuclear cells to rapidly accumulate at the point of injection. Recent studies have also shown that extracts prepared from normally evolving delayed hypersensitivity skin sites contain biologically active materials when tested *in vivo* and *in vitro*(70). For example, when these soluble extracts are injected intraperitoneally into normal animals the macrophage content of exudates recovered from these animals are increased. They also induce a mononuclear infiltrate when injected into the skin of normal animals. In addition, these materials contained chemotactic activity for macrophages and lymphocytes when assayed *in vitro* but did not possess MIF activity. Extracts prepared from the sites of arthus reactions only contained chemotactic activity for neutrophils.

The recovery of substances having mediator activity under conditions where immunologic processess are occuring adds further evidence in favor of their pathogenetic significance. In this regard, it was of interest that substances having MIF, chemotactic and mitogenic activities have been detected in cultures of lymphocytes recovered from a patient undergoing an allograft rejection(71). Lymphocytes isolated from the rejected kidney

were cultured in vitro without further antigenic stimulation and the supernatants assayed for mediator activity. Activities for the above named factors were present in significant amounts in the culture supernatants as well as a procoagulant material (tissue factor) which shortened the clotting time of normal plasma. Supernatants obtained from peripheral blood lymphocytes or irradiated kidney lymphocytes cultured in parallel did not contain significant mediator activity. These findings suggest that perhaps the cells isolated from the kidney were involved in its rejection.

Since the usual means for eliciting mediator production is by culturing lymphocytes in vitro, it was of interest to learn that MIF and interferon activity could be detected in serum obtained from animals(72). Serum collected from mice immunized with Bacillus Calmette-Guerin and then desensitized by receiving a large dose of Old Tuberculin antigen intravenously was found to contain MIF and interferon activities. When this serum was filtered on Sephadex G-100 gels both activities were found in the same fraction (MW 45,000).

Willoughby and co-workers(13) have described a vasoactive material recoverable from guinea pig lymph node cells that increases vascular permeability within 30 minutes after injection into normal skin. This material, termed lymph node permeability factor(LNPF), was extracted from lymph nodes of immunized animals but could also be obtained in equal amounts from non-immune animals. Extracts prepared from other tissues including spleen, thyroid, lung, kidney, liver and muscle also contained LNPF activity. This activity could be differentiated from other known permeability factors such as histamine, 5-OH-trypamine, bradykinnin and Kallikrein. LNPF was found to retain its activity between pH 5-9, was stable at 100°C for 30 minutes, and was excluded from Sephadex gels(G-100) indicating a MW greater than 100,000(74). It was also shown to be resistant to digestion by RNAse, DNAse, trypsin, chymotrypsin and papain although it was inactivated by pronase. LNPF appears to be different in many respects from the other mediators already discussed. It is released without an immunologic stimulus and is found in numerous tissues suggesting that it may not play a primary role in the delayed hypersensitivity reaction. It may, however, play a secondary role by contributing to the inflammatory process per se through its release subsequent to cell or tissue damage. Of interest is the observation that skin reactive factor prepared by immunologic activation of lymphocytes also seems to possess vasoactive properties(75). This material(MW 39,000) induces the extravasation of Evans blue dye between 20 minutes and 4 hrs following its intradermal injection. The increased permeability induced by SRF was not blocked by inhibitors of histamine or 5-OH-trypamine but was affected by inhibitors of the kinnin system. It is possible that SRF and LNPF mediate their effects through different pathways.

Pharmacologic Modulation of Mediator Activity - It is well known that cyclic 3'5'- adenosine monophosphate(C-AMP) plays an important role as a "second messenger" in the regulation of intra-cellular metabolism. The possibility that C-AMP is involved in the expression of biologic activity of mediators such as MIF on their target cells or on lymphocyte mediated

cytolysis has been recently investigated. It has been shown that immunologic reactions such as lymphocyte mediated cytotoxicity and the inhibition of macrophage migration by MIF are abbrogated by agents which raise C-AMP levels. This is in contrast to normal secretory mechanisms where increased levels of C-AMP result in increased production of cell products. For example, drugs such as isoproterenol(10-3M), epinephrine(10-4M), and prostaglandin E_1(5 ug/ml) which raise C-AMP levels by stimulating adenyl cyclase and theophylline(10-4M) which retards its breakdown, interfere with the MIF induced inhibition of guinea pig macrophages from capillary tubes(76). The effect appears to be on the macrophage response to MIF and not due to inactivation of MIF by the drugs. The dibutyryl derivative of C-AMP is also effective in blocking MIF activity. There is preliminary data to suggest that isoproterenol, theophylline and dibutyryl C-AMP may also block the production of MIF by lymphocytes(76). In related studies C-AMP levels in macrophages have been shown to be lowered in the first 24 hrs of incubation with MIF(77).

Cell-mediated cytotoxicity by sensitized mouse lymphocytes on mastocytoma target cells is also prevented by agents which raise C-AMP levels. Thus isoproterenol, theophylline, histamine, prostaglandin E_1 and E_2 and dibutyryl C-AMP are all effective in inhibiting this immunologic reaction (78). Furthermore, propranolol reverses the inhibitory effect of isoproterenol on cytotoxicity. C-AMP levels in the effector lymphocytes were shown to be elevated following exposure to these drugs. Other workers have made similar observations(79). In this latter study prostaglandin E_1 cholera toxin and theophylline were all effective in abbrogating the cytotoxic response of sensitized lymphocytes in the mouse system. Of interest was the finding that cholinergic agents enhanced the amount of cell lysis, possibly by raising cyclic guanosine monophosphate (C-GMP) levels. This enhancement of cytotoxicity could be blocked by atropine. These studies indicate that the secretory processes of lymphocytes involved in immunologic reactions are modulated by cyclic nucleotides such as C-AMP and C-GMP.

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Section VI - Topics in Chemistry

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Chapter 30. Medicinal Inorganic Chemistry

Robert P. Hanzlik, Department of Medicinal Chemistry
University of Kansas, Lawrence, Kansas 66044

In recent years the demarcations between the various classical divisions of chemistry, i.e. organic vs. inorganic vs. biochemistry, have become more and more diffuse. In particular, the interfacing of inorganic chemistry with the biological sciences has provided a very fertile area. The spreading momentum of this interdiscipline is reflected in a number of recent reviews and books.[1-4]

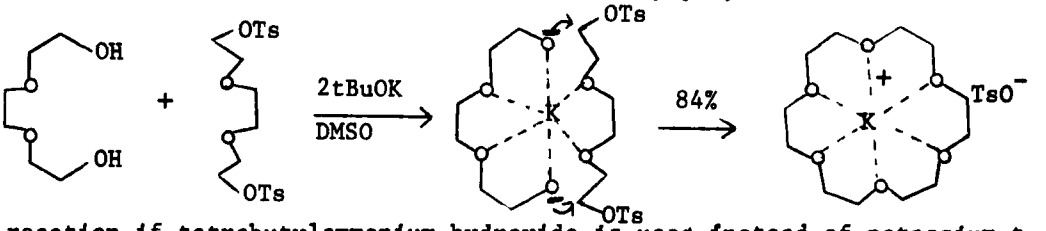
This report will deal with the inorganic aspects of physiological and medicinal chemistry, and with the practical use of inorganic chemistry by organic chemists as a "tool" in the laboratory. (While these areas may seem distinct it is well to remember that the same chemical principles define the scopes and limitations of both.)

The chemistry and biochemistry of alkali (group Ia) and alkaline earth (group IIa) metals have received considerable attention since the discovery of the crown ethers[5,6] and the inophorous antibiotics.[7] Prior to these discoveries the paucity of stable complexes was the outstanding feature of the chemistry of the Ia and IIa cations, and it contrasted sharply to the chemistry of transition metal ions. A wealth of data now confirm that the solution behavior and complexing tendencies of the Ia and Ia ions, as well as their solid state behavior, is determined mainly by the size of the ion, or more accurately the charge/size ratio of the ion. Complexation is an equilibrium process favored by large negative enthalpy and large positive entropy contributions to the free energy of the process. Cations with a high charge and small size will attract anions or negative dipoles more strongly than larger ions with lower charge. Consequently small ions such as Li^{+}, Be^{++}, Mg^{++}, and Al^{+++} tend to form strong bonds with considerable covalent character. The organic chemistry of alkyllithium or Grignard reagents reflects the polarization of an ionic bond (R^{-}, M^{+}) into a more or less covalent bond (R - M) by the high electrostatic charge density on the small cation.

In some cases, entropy effects control the complexation behavior of Ia and IIa cations. A macromolecular ligand may free many individual solvent molecules when it complexes with a cation, thereby creating very large positive entropy contributions. This is nicely illustrated by the finding[8] that the equilibrium constants for solvent-separation of the 9-fluorenylsodium ion pair in tetrahydrofuran by a series of glymes, $CH_3O(CH_2CH_2O)_nOCH_3$, increased from 1.4 when n=2 to 800 when n=6. Calorimetric studies indicated only very small enthalpy changes, which implies

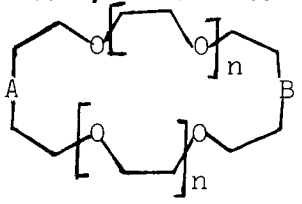
that the entropy effects of chelation alone are responsible for driving the reaction.

A very important feature of such polydentate chelation has become known as the "template effect," and is illustrated here by a synthesis of a crown ether complex.[9] The fact that only polymer forms in a slow

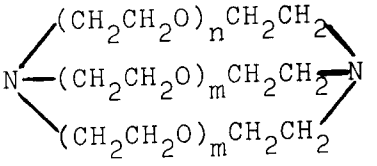


reaction if tetrabutylammonium hydroxide is used instead of potassium t-butoxide suggests that the potassium ion serves to gather the loose ends of the reactants thereby making the cyclization an essentially intramolecular process.

Crown ethers, Cryptates, Antibiotics, and Aspirin - The crown ethers, the cryptate or "football" ligands, and the antibiotics valinomycin, gramicidin-A, nigericin, X-537A, monesin, enniatin B, nonactin, monactin, dinactin, and trinactin, all have in common the ability to form strong



A,B = O, NH, S
n = 1,2,...



(m or n = 1 or 2)

complexes with Ia and IIa cations, and with certain others such as NH^+, silver (I), and thallous ion. They all possess or can fold up to form a large ring of 14 or more members including a more or less symmetrical arrangement of -CO-, -NH-, -O-, or -S- groups in the ring which serve to bond to the metal. The x-ray structures of many of these and related complexes have been reported.[10]

One outstanding feature of these ligands is their ability to dissolve or extract ionic salts such as KCl into an organic phase. This very useful property makes possible the preparation of benzene solutions of K(crown)OH[11] and K(crown)MnO_4, "purple benzene",[12] for clean and fast homogeneous reactions with organic molecules. The crown ligands also hold considerable promise for use with Grignard and alkyllithium reagents (much as $Me_2NCH_2CH_2NMe_2$ is now used with RLi reagents), for use in nonaqueous electrochemistry and for use in modifying dissolving metal reductions, although these areas are still largely unexplored.

Another outstanding feature of this group of ligands is their selectivity in complexation of ions. Discrimination is according to the relative sizes of the ligand cavity vs. the ion. Selectivity with a given

ligand is high in methanol and low in water because water is a better competitor than the crowns for solvating the cations. The cryptate and antibiotic complexes are usually stronger than crown complexes.[13] Consequently the cryptate ligands and antibiotics are even more cation-selective than the crown ethers.

Biological Action and Ion Carriers - There is a high potential energy barrier to the passage of an ion from one aqueous phase, through a lipid membrane, into another aqueous phase. Two theories to explain the facile transport of ions observed e.g. across nerve membranes are known as the "carrier" hypothesis and the "pore" hypothesis. In the carrier model a molecule within the thickness of the membrane picks up a cation at one surface, diffuses through the membrane, releases the ion at the other surface and returns empty, like a shuttlebus. The pore model pictures channels or tubes through the thickness of the membrane which ions could use to "tunnel" through the potential barrier. Experimental support has been found for both of these theories, but the work has been confined to artificial membranes for the most part.[7]

In the simplest experimental systems, valinomycin[14] and nigericin[15] have been found to transport potassium ions across a chloroform barrier in the bottom of U-tube in response to a potassium ion concentration differential between aqueous layers in the arms of the U-tube. The chemical potential of an anion concentration gradient between the two arms can also drive (or inhibit) the transport of potassium. These systems display saturation kinetics at high potassium concentrations where it is difficult for the carrier to unload. Thus they model important features of the biological systems.

More sophisticated experiments with phospholipid bilayers, thought by some to be synthetic or model membranes, also demonstrate the ease with which carrier molecules can transport ions through lipid membranes.[7,16] In these experiments a phospholipid bilayer ca. 70 A. thick separates two aqueous solutions of ions and creates a very low electrical conductance. When crown ethers or ionophorous antibiotics are "dissolved" in the lipid bilayer the conductance increases linearly with the concentration of "carrier". The conductance also increases linearly with potassium concentration but again a saturation plateau is reached at high concentrations. These results are taken to mean that a 1:1 K^+: carrier complex is responsible for the conductance, rather than the carriers stacking up to form a tube or pore through the membrane. Although the carrier hypothesis is an old one and natural carrier molecules have yet to be identified, the above experiments and others reviewed by Läuger[16] lend credence to the carrier hypothesis.

The feasibility of ionic conduction through membranes via pores is also an experimental fact. An antibotic provides the model, but again no natural pores in membranes have been identified. Gramicidin A, a polypeptide of M.W. 1880, increases the conductance of phospholipid membranes. It is suggested that the peptide coils into a helical structure with a hollow central channel about 4A in diameter. Conductance mediated by

gramicidin A is far too efficient for a shuttlebus mechanism and a pore model best reconciles the data with calculations.[7]

In addition to passing through membranes, metal ions are also components of membranes. They may neutralize excess negative charge on the membrane or bind various components to give structural integrity to the membrane. The binding of metals such as calcium to artificial phosphatidylserine bilayers reduces the nagative surface charge on the membrane. As the surface charge becomes less negative the approach of a cation from an aqueous phase becomes energetically less favorable. As expected, calcium ion decreases the nonactin mediated transport of potassium through phosphatidylserine membranes. Conversely, the approach of anions is facilitated by a less-negative surface charge, and transport of I_5^- through phosphatidylserine is enhanced by calcium ions.[7] An interesting extension of this concept involves the effects of salicylates on the ionic conductance of human erythrocyte and molluscan neuronal membranes.[17] With the molluscan nerve membrane the normal relative permeability of K^+ is 1.0:0.06. Absolute conductance measurements in the presence of salicylates indicate a large increase in K^+ conductance, a small increase in Na^+ conductance, and a decrease in Cl^- conductance. The effects were dependent on the dose of the salicylate and for a variety of salicylates a correlation was found between the magnitude of the permeability effect and the octanol-water partition coefficient. The authors propose that an alteration of the net surface charge on the membrane, as a result of the salicylate dissolving into it, "is the basis for the analgesic action of salicylate".[18] The anti-inflammatory action of salicylates, and an indirect reduction in pain, is presumably related to inhibition of prostaglandin biosynthesis or release.[19,20]

The hexaamminecobalt (III) ion, $Co(NH_3)_6^{+3}$, which has been found to inhibit the transport of calcium across mitochondrial membranes[21] is a good illustration of a fundamental difference between some transition metal ions and the Ia/IIa cations. The latter all tend to be highly labile toward substitution of one ligand for another, while some of the former, particularly d^3 and d^6 ions such as Cr(III) and Co(III), tend to be kinetically inert toward ligand exchange. $Co(NH_3)_6^{+3}$ is quite stable in aqueous solution over a considerable range of pH; otherwise it would be pointless to discuss this ion in a biological system.

Special Properties of Transition Metals - Many of the biological and abiological reactions involving metals have been reviewed,[22-26] but some recent examples serve nicely to illustrate the scope of metal involvement in reactions of interest. They also illustrate the intrinsic and unique properties that transition metals contribute to the systems.

Optical Activity: The best known optically active organic catalysts are enzymes. They discriminate between enantiomers of substrates and can determine right- and left-handedness in meso substrates such as citric acid. It is not generally appreciated that the inter-conversion of citrate (meso) with isomerically pure cis-aconitate and threo-D-(2R,3S)-isocitrate is a result of coordination of the reactants on one face of an

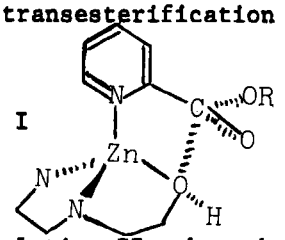
octahedral Fe(II) in the enzyme aconitase.[27] An optically active metal chelate was found to decarboxylate meso α-methyl-α-aminomalonic acid to give alanine with 25-30% enrichment of the L-isomer.[28] Nearly all attempts to confer optical activity to products from racemic or meso substrates by use of optically active reagents are far short of the perfection of most enzymes. A stark exception to this rule was reported recently. A rhodium hydrogenation catalyst having chiral phosphine ligands was resolved into its optically pure isomers and using the resolved catalyst it was possible to synthesize 90% optically pure L-amino acids by reducing a meso precursor compound.[29] Resolving a pound of catalyst rather than tons of product should facilitate manufacture of L-dopa, an anti-Parkonsonism agent, and L-phenylalanine, a potential low-calorie sweetener. An interesting resolution of amino acids based on a transition metal complex has been reported recently.[30] A racemic amino acid is absorbed by a resin containing an optically active Cu(II) chelate, and the isomer bound less strongly (usually D) is displace by ethylenediamine.

Template Effects: Template or ligand gathering effects have been used not infrequently in organic chemistry. Examples include the use of "methylmagnesium carbonate" for carbomethoxylation of active methylenes,[31] the use of zinc ion in controlling stereochemistry in aldol condensations[32] (yeast aldolase also uses zinc at the catalytic site),[33] and in the synthesis of corrins.[34] Other examples have been given in previous reviews. Recently an elegant example of metal ion stabilization of protein tertiary structure has emerged from studies of concanavalin A,[35] a tetrameric protein from the Jack bean which binds carbonhydrates in the fashion of an antigen-antibody complex.[36] It has the unique feature of binding to glycoproteins on the surfaces of cells transformed by DNA tumor viruses or chemical carcinogens, but it does not bind to normal cell surfaces. Before carbonhydrate binding can occur, each Con A subunit must bind, in succession, a manganous ion and a calcium ion. The crystallographic space group, and presumably the solution conformation, changes as a result of metal ion binding.[35] Manganous ion has a distinct preference for octahedral coordination and probably serves to "generate" the Ca^{++} site by ligand gathering. Calcium, typical of Ia and IIa ions, has more flexible coordination geometry, and fits into an irregular pentacoordinate site of high negative charge. Since this unique "double site" is located about 22 A from the carbonhydrate binding site, the metals must affect carbohydrate binding through long range conformational (allosteric) effects.

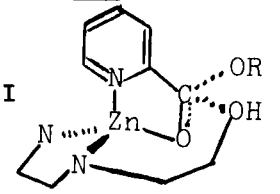
Probe Properties: The use of metal ions as probes of molecular environments by virture of their spectral or magnetic properties has been reviewed.[37]

Lewis Acidity: Transition metal ions are moderate to soft Lewis acids and most ligands are Lewis bases. From this one would a priori predict that a coordinated nucleophile would be less reactive than a free nucleophile. However, evidence that coordinated nucleophiles can have appreciable reactivity has been accumulating. The generally accepted mechanism for carbonic anhydrase action envisions activation of water by coordina-

tion to Zn(II) with loss of a proton. The result, at neutral pH, is an active hydroxide which attacks the neutral CO_2 molecule to produce bicarbonate.[38] A simpler example of the same type of process may be a reported[39] transesterification thought to proceed via complex I. An alter-

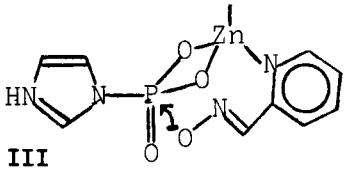


I

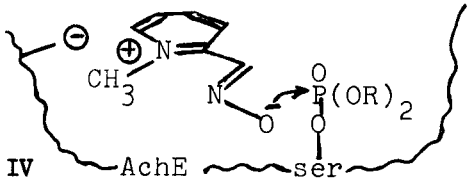


II

nate formulation II makes the transition state more like that of carboxypeptidase A, i.e. enhancement of carbonyl electrophilicity rather than hydroxyl nucleophilicity.[40,41] Detailed studies of hydrolysis of peptides and amino acid esters by Co(III) complexes have shown that both type I and type II mechanisms operate under various conditions.[42] Another interesting zinc catalyzed transesterification has been proposed as a model for the nucleoside diphosphokinase reaction, ATP + GDP ⇌ ADP + GTP. The mechanism involves two steps with a phosphorylated enzyme histidine intermediate. In the model, III, the oxime substitutes for the nucleoside diphosphate in the role of phosphate acceptor.[43] The reaction is facilitated by template and Lewis acid effects. There is a certain similarity



III

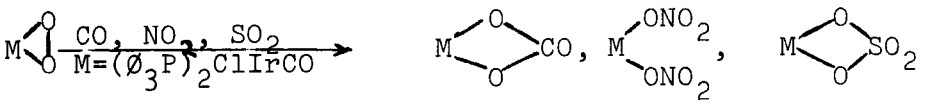


IV

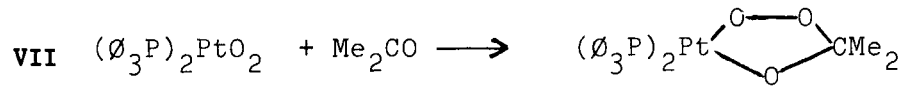
between this process and the reactivation of a phosphorylated active site of acetylcholinesterase IV by pralidoxime (shown) or 1-methylnicotinic hydroxamic acid.

Redox Properties: It is probably an understatement to say that 80% of biological and synthetic oxidations and reductions depend on metal species as reagents, catalysts, or active site components. Therefore this discussion is arbitrarily restricted to oxygen complexation and/or activation by chemical and biological systems. There has been a great deal of effort spend in investigatigating both mixed function oxidases and cytochrome P_{450}, and numerous reviews have been written.[44-48] However, few of these have actually addressed the question of the nature of enzymatically activated oxygen and the mechanism of the atom transfer step.

In the past few years great advances have been made in studying the reversible binding of oxygen to inorganic compounds. Bound oxygen clearly has different properties from free oxygen and some of the complexes undergo further irreversible or catalytic chemistry. The type VII[49,50] and perhaps the type VI[51,52] reactions are consistent with considering the coordinated oxygen to have the form M^{+2}-O_2^{-2}, i.e. nucleophilic coordinated peroxide. The type V and perhaps the type VI reactions may involve coordination of the O-acceptor prior to O-transfer. With type VI systems,

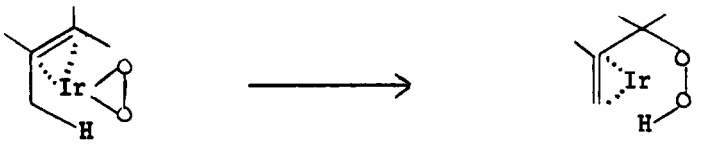
V 

VI $O_2 + 2X \xrightarrow[M=Ni,Pt,Pd]{(Ø_3P)_2MO_2} 2\ XO$, $X = Ø_3P$, RNC

VII 

VIII $(Ø_3P)_4M + O_2 \longrightarrow (Ø_3P)_2MO_2$, $M = Ni, Pd, Pt$

olefins are not effective O-acceptors and autoxidations catalyzed by type V complexes[53] are probably free radical in nature. Hydroperoxides are the primary products which secondarily decomplse to produce epoxides and alcohols, or unsaturated ketones. However an interesting interpretation of hydroperoxide formation views the reaction as an "ene" reaction of singlet oxygen and olefin within the coordination sphere of the diamagnetic complex.[55] Singlet oxygen has been postulated in enzymatic hydroperoxidation and chemical evidence implicating its generation by enzymes

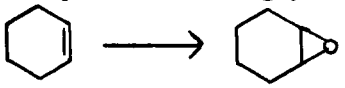


has been obtained.[56] However enzymatic studies have shown that the oxygen enters and the hydrogen departs from opposite sides of the allyl plane, contrary to the concerted cyclic mechanism pictured for singlet oxygen.[57] The Cr(VI) complex $EtO{\cdot}CrO(O_2)_2$ was proposed to furnish singlet O_2 but a reinvestigation implicated traces of H_2CrO_4 impurity as the oxidant.[58] A related Cr(V) complex K_3CrO_8 reacts with olefins giving the same product distribution as singlet oxygen.[59]

Other metals in high oxidation states containing oxo or peroxo groups oxygenate olefins. This group includes MnO_4^-, RuO_4, and OsO_4, and those non-catalytic examples given below.

$(HMPA)MoO(O_2)_2$ + 2C=C → 2C(O)C — ref. 60

$FeO_4^=$ + C=C → epoxide and/or glycol — ref. 61

CrO_2Cl_2 +  — ref. 62

Superoxide anion is a popular candidate for many enzymatic oxidation reactions, and superoxide dismutase is being persued as a test for the intermediacy of free superoxide as well as for its own interesting properties. Coordinated superoxide may be a more attractive candidate as an enzymic intermediate, and recently it has been possible to isolate Co(III) superoxide complexes and to study them by physical methods (IR,

ESR, and photoelectron spectroscopy).[64-66] A large number of Co(II) compounds form O_2 adducts formulated as $Co(III)O_2^-$. Usually these complexes are reactive and binuclear peroxides M-O-O-M are ultimately formed. Some of these complexes are formed reversibly, as are certain Ir complexes analogous to type V.[67]

There are two reports that Co(III) compounds related to the oxygen carriers can introduce oxygen into organic compounds; both cases involve phenols as O-acceptors.[68,69] Weak and irreproducible catalysis of olefin oxidation has been observed with similar compounds.[70] Other Co(II) chelates potentially capable of forming superoxide adducts are highly efficient catalysts for organo-phosphine oxidation.[71]

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Chapter 31: Reactions of Interest in Medicinal Chemistry

Herbert T. Nagasawa and Dwight S. Fullerton
Veterans Administration Hospital, and
College of Pharmacy, University of Minnesota,
Minneapolis, Minnesota 55417

The recent publication of the following useful reference books on the synthetic applications of organic reactions should be noted: the completely revised edition of Modern Synthetic Reactions by H.O. House, Volume 3 of Reagents in Organic Synthesis by Mary Fieser and Louis F. Fieser, Compendium of Organic Synthetic Methods by I.T. Harrison and S. Harrison, and Annual Reports in Organic Synthesis, 1971 by J. McMurry and R.B. Miller. The journal Synthesis continues to abstract interesting synthetic reactions selected from the world literature (about 800 abstracts in 1972).

Review articles will be cited in the appropriate sections. Because of space limitations, the reactions actually presented are necessarily highly selective.

Computers - Advances in the use of computers for the design of synthetic schemes have been described by E.J. Corey and coworkers.[1,2,3,4] The application of computer graphics to input and output chemical structures, the development of a computer program to effectively perceive structural units and relationships, and the use of a computer system to generate a "tree" of possible intermediates starting from a given target molecule are discussed.

Useful Reactions (not elsewhere classified) - The facile use of *stereoselective hydrocyanation* has been described.[5,6,7] Since the nitrile function can be converted to methyl as well as to a variety of functional groups, the conjugate addition of HCN has wide application. The use of *organosulfur compounds* in organic syntheses has also been reviewed[8] and covers most of the literature since 1955.

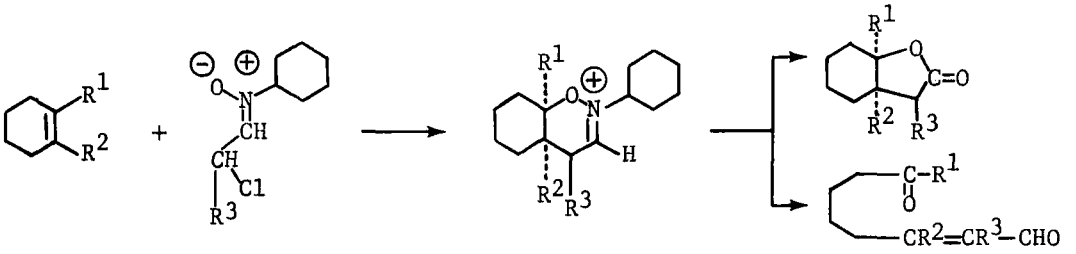
Organic *reagents linked to polymeric resins* have been prepared to simplify reaction work-up. Resins containing Wittig reagents have been reported to eliminate the problem of phosphine oxide removal from the crude products.[9] Similarly, the use of carbodiimide linked to polystyrene has solved the problem of urea removal.[10]

The bicyclic amidines 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) have proven to be superior to pyridine, N,N-dimethylaniline, and other common amines in *dehydrohalogenations* of very sensitive molecules. A review summarizes their use in dehydrohalogenations, condensations, and rearrangements.[11]

Modifications of the *Claisen rearrangement* have been used to introduce angular methyl groups,[12] to synthesize unsaturated aldehydes (by

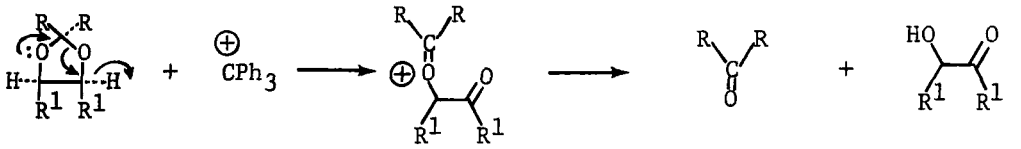
sequential Claisen and Cope rearrangements),[13] to convert ene-thiols to a variety of products including derivatives of thiophene and 2H-thiopyran,[14] and to convert allyl esters to the corresponding γ,δ-unsaturated acids under very mild conditions.[15]

α-Chloronitrones have been explored as "enophiles" that would undergo *4+2 cycloadditions* with isolated olefinic double bonds, even though the double bond is not activated.[16,17,18] The α-chloronitrone adduct can be converted in high yields to useful synthetic intermediates.



Protective Groups and Their Removal - BF_3-etherate is often better than TsOH for catalyzing the rapid formation of *O-tetrahydropyranyl* derivatives of alcohols.[19] The *dimethyl-tert-butylsiloxy group* which is 10^4 times more stable than trimethylsiloxy may be used as an alternate to the tetrahydropyranyl group for protection of alcohols.[20] The absence of a chiral center and its stability to catalytic hydrogenolysis or to mild chemical reducing agents, as well as to conditions of the Wittig reaction or the Jones (CrO_3) oxidation, offer wide latitudes for potential application. The dimethyl-tert-butylsiloxy ethers are rapidly cleaved to alcohols with 2-3 equivalents of tetra-n-butylammonium fluoride in THF or with HOAc-H_2O (2:1) at room temperatures.

Very sensitive *acetals* can be oxidatively cleaved with triphenylcarbonium ion.[21] (Conversely, the acetonide of a diol can be oxidized to

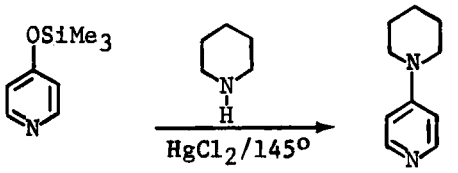


an α-hydroxyketone by this method.) Ketones or aldehydes protected as *thioketals* or *thioacetals* are readily regenerated by conversion to the reactive sulfonium salts with Meerwein's reagent[22] ($Et_3\overset{+}{O}\overset{-}{BF_4}$) or methyl iodide in moist acetone,[23] followed by hydrolysis. Dialkyl and phenyl ethyl *ethers* are cleaved under essentially neutral conditions with triphenyldibromophosphorane, Ph_3PBr_2.[24]

The *α-picolinyl group*, which can be selectively removed by mild acid hydrolysis in the presence of Cu(II) ions, has been used as an amino protecting group in peptide synthesis.[25] o-Hydroxysubstituted *aromatic ketimines* of amino acids have also been utilized in this manner.[26] The pro-

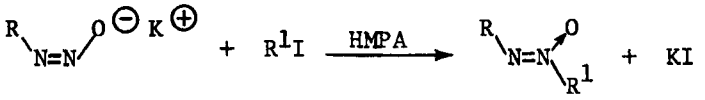
tective group is removed with 80% HOAc at 80° -- conditions which leave the t-BOC group intact. A protective group that is stable to acid or alkaline treatment but which is cleaved by treatment with Ac_2O at room temperature is the *1-oxy-2-picolyl* (OP) group.[27]

Functional Group Transformations - Phenols are smoothly converted to arylamines *via* 4-aryloxy-2-phenylquinazolines which are thermally rearranged to 3-aryl-2-phenyl-4(3)-quinazolinones, then hydrolyzed.[28] *Anilines* can also be prepared from phenols by amination of their diethylphosphate esters with KNH_2.[29] *Trimethylsilyl ethers* of aromatic hydroxy nitrogen heterocycles, including purines and their protected nucleosides, can be *aminated* with NH_3 or 1° and 2° amines in the presence of 0.1-0.2 equivalents of Lewis acid.[30,31]



Stereospecific conversion of alcohols to amines with essentially complete *inversion* of configuration has been effected by reacting the alcohol with 1 equivalent each of diethyl azodicarboxylate, Ph_3P, and phthalimide (or siccinimide).[32] The product is an N-alkylphthalimide from which the amine can be liberated with hydrazine-hydrate. 1° and 2° allylic alcohols have been converted to the corresponding *unrearranged* chloride with high specificity using Ph_3P-CCl_4.[33]

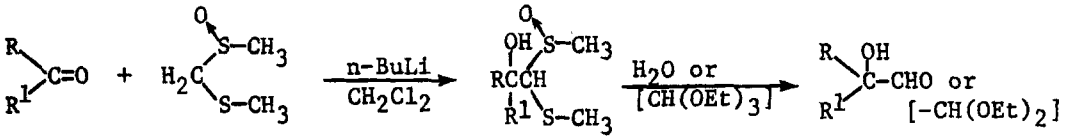
Reductive dechlorination of organic halides with $LiAlH_4$ appears to be a general reaction that can be extended to the hydrogenolysis of vinyl, bridgehead and cyclopropyl halides.[34] A *modified Curtius degradation* utilizes trimethylsilyl azide, which is nonhazardous by virtue of its thermal stability.[35] A flexible and directed synthesis of unsymmetrical *trans-azoxyalkanes* which involves the alkylation of alkyl diazotates has been described.[36]



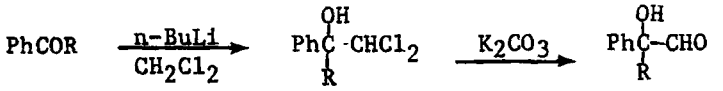
5-Fluorouracil has been prepared directly from uracil in 85% yield by *electrophilic fluorination* with CF_3OF.[37] Similarly, adenosine, AMP, and ADP can be chlorinated directly to the *8-chloronucleoside or nucleotide* with the reagent, tetrabutylammonium iodotetrachloride $(n\text{-}C_4H_9)_4NICl_4$.[38] This procedure is amenable for the preparation of the ^{14}C-labeled 8-chloronucleoside(tide).

An elegant method for the conversion of nucleosides to their *5'-deoxy derivatives* involves the transformation of the primary 5'-hydroxy groups to nucleoside 5'-(pyrimidine-2-yl-thio)-5'-deoxy derivatives followed by Ra-Ni desulfuration.[39] Unprotected purine and pyrimidine ribonucleosides react with ethyl trichloromethanephosphonate $[Cl_3CP(O)(OEt)_2]$ in the presence of Et_3N to give *2'(3')-ribonucleotide ethyl esters* which can be transformed directly to *2'(3')-nucleotides* by treatment with alkali.[40]

Aldehydes and Ketones - The transamination of amines using benzothiazole-2-carboxaldehyde and a base appears to be a useful method for preparing aldehydes and ketones.[41] Reaction of carbonyl compounds with the easily prepared[42] lithio derivative of methyl (methylthiomethyl) sulfoxide followed by hydrolysis or solvolysis with ethyl orthoformate gives, respec-



tively, α-*hydroxyaldehydes* or α-*hydroxyaldehyde diethylacetals*.[43] *Alpha*-hydroxyaldehydes can also be prepared by hydrolysis of the dichloromethyl carbinols produced when alkyl aryl ketones are reacted with dichloromethyl lithium in THF at -100°.[44]

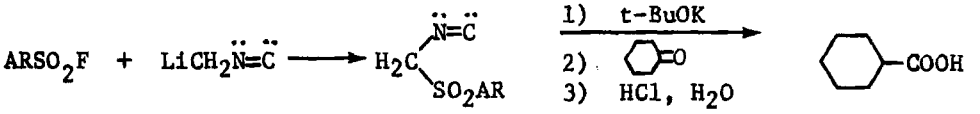


Lithio derivatives of tetrahydropyranyl-protected propagylic alcohols can be converted *via* protected allenic ethers to α,β-*unsaturated aldehydes*.[45]

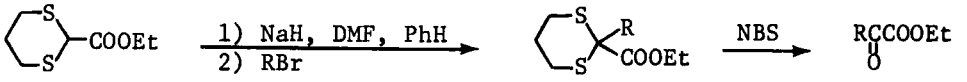
Aryl ketones have been produced from acid chlorides and arenes with only catalytic amounts (1%) of CF_3SO_3H.[46] Friedel-Crafts acylations using trace amounts of non-Friedel-Crafts catalysts (iron, copper, iodine, metal halides, acids) have been summarized.[47]

Carboxylic Acids and Derivatives. - *Quarternary ammonium salts* of carboxylic acids (unlike Ag, Pb, alkali metal, or tertiary amine salts) are converted to their *esters* at room temperatures in dipolar aprotic solvents even with relatively unreactive halides.[48] *Sterically-hindered* carboxylic acids (strong acids included) can be esterified with dimethyl sulfate in the presence of aqueous or anhydrous alkali since the reaction center is two atoms removed from the bulky R group.[49]

Carbonyl compounds are converted to the *next higher carboxylic acids* by reaction with α-metalated isocyanomethyl aryl sulfones followed by hydrolysis of oxazoline intermediates.[50]

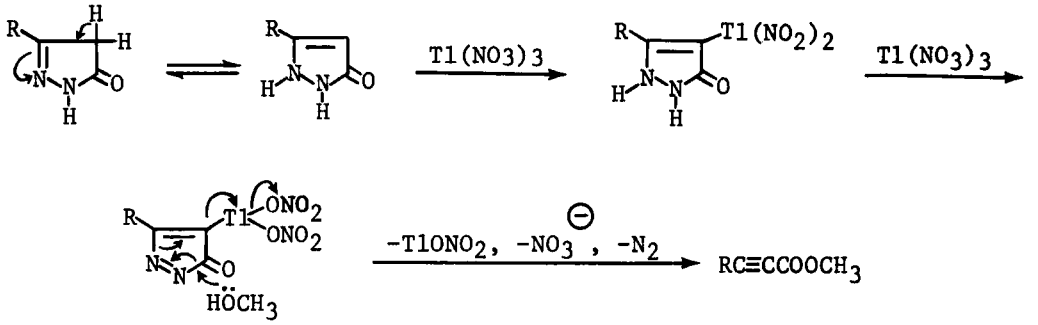


As shown on the next page, α-*keto esters* are produced when the readily available 2-carbethoxy-1,3-dithiane is alkylated and the product oxidatively dethiated.[51] By bromination of α-alkylacetoacetates and subjecting the α-bromo-α-alkylacetoacetates to a Japp-Klingemann reaction with



base, α-*monobromo esters* and α-*monobromo acids* can be prepared.[52] A new procedure for the Darzens synthesis of *glycidic esters* is based on prior generation of the α-haloester anion with lithium *bis*(trimethylsilyl)amide in THF at -70°.[53] *Propargylic esters* can be prepared in good yield from trialkynylborane and ethyl diazoacetate.[54] Treatment of α-diazo-β-hydroxy-esters or ketones (prepared by the condensation of aldehydes with ethyl diazoacetate or acyl diazomethane) with BF_3-Et_2O-CH_3CN solution gives *acetylenic esters* or *ketones*.[55] High yields of *2-alkynoic esters* are produced from 5-pyrazolones (prepared from β-keto esters and hydrazine) by

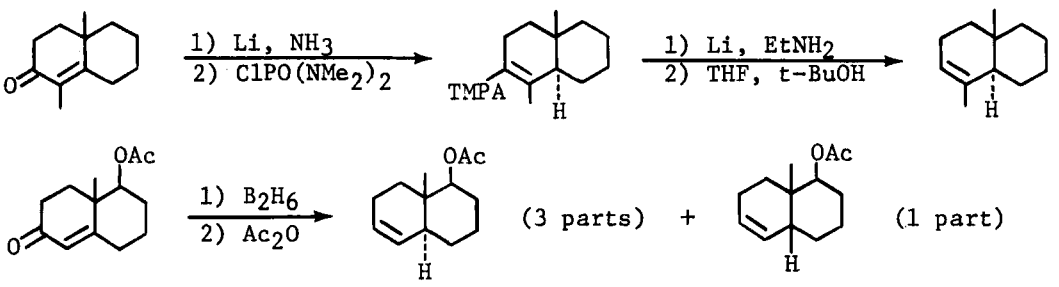
reaction with thallium(III)nitrate in methanol.[56]



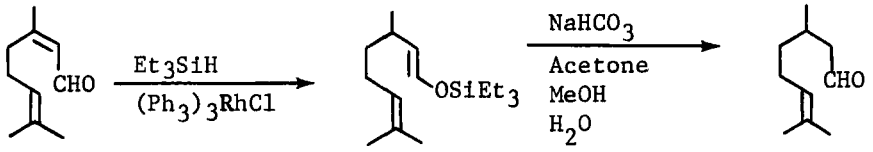
Primary aliphatic, olefinic and aromatic mono- and diamides have been converted to *nitriles* in >90% yields by trimethylsilylation of the nitrogen followed by treatment of the TMS derivatives with acetyl or benzoyl chloride.[57] Nitriles can also be prepared in 70-90% yields by treating aldoximes with cyanuric chloride in pyridine.[58] Addition of HCN to the tosylhydrazines of ketones gives 1,2-addition products, which on thermolysis, likewise yield nitriles.[59]

Oxidations, Reductions, and Epoxidations - Because of its lower vapor pressure, *potassium osmate* is a much safer alternative to osmium tetroxide for making *1,2-diols*.[60] Double bonds which normally do not react with m-chloroperbenzoic acid can be made to react in inert solvents at elevated temperatures in the presence of radical inhibitors to yield *epoxides*.[61]

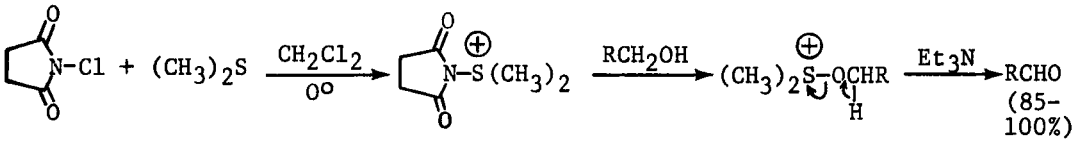
Alcohols or enols may be protected as diethylphosphates (DEP) or tetramethylphosphordiamidates and *reductively deoxygenated* in lithium ethylamine solution.[62] In some cases, this reaction has some advantages over the Wolff-Kishner reduction. Enones may also be deoxygenated to the isomeric olefins by hydroboration.[63]



Selective reduction of the conjugated double bond of α,β-unsaturated aldehydes and ketones has been accomplished with "rhodium complex" and triethylsilane.[64]

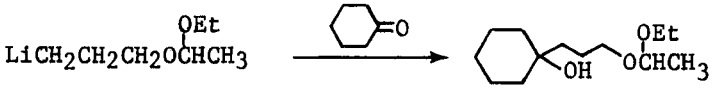


A mild method for the *oxidation of alcohols* to ketones or aldehydes should be useful with very sensitive molecules.[65] There is little variation in optimal conditions.



The numerous uses of alkyl aluminum hydrides and alkoxyaluminum hydrides have been reviewed.[66] Sodium hydrogen sulfite (but not sodium sulfite) readily reduces *sulfoxides to the corresponding sulfides*. If the sulfoxides are converted to alkoxysulfonium salts, sodium sulfite will also accomplish the reduction quantitatively.[67] Amines may be *reductively methylated* with formaldehyde and sodium cyanoborohydride in acetonitrile.[68] The mild conditions, high yields, and ease of experimental manipulation make this a method of choice.

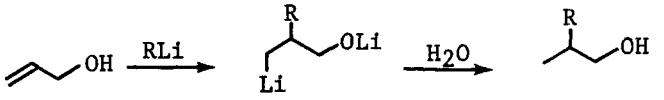
C-C and C=C Bond Formation - Organolithio reagents prepared from ethyl 3-bromopropylacetaldehyde acetal are useful for the introduction of the *hydroxypropyl group*.[69] The reagents are stable for several months at -30°. The organolithio reagent can also be converted to the corresponding lithio organocuprate.



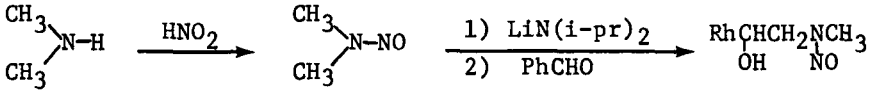
A modified Hooz reaction which involves the reaction of ethyl diazoacetate with dialkyl chloroboranes results in a *two carbon homologated* product.[70] Allyl alcohol adds a variety of organolithium reagents

$$R_2BCl + N_2CHCOOEt \xrightarrow{-78^{\circ}} \xrightarrow{MeOH} RCH_2COOEt + RB(OCH_3)_2$$

regiospecifically to give 2-substituted 1-propanols.[71]

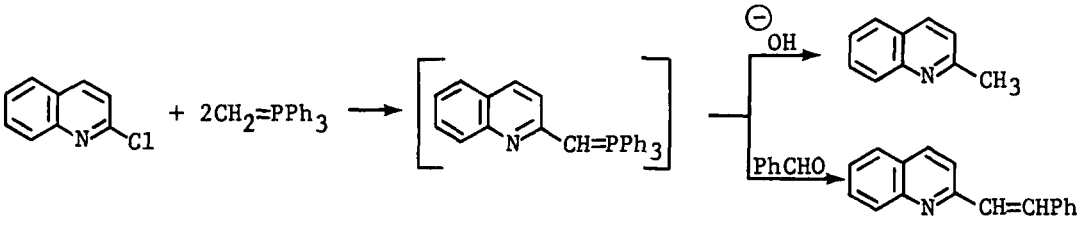


The *direction of alkylation* of α,β-unsaturated esters has been studied. The enolate ions generated with lithium N-isopropylcyclohexamide in THF at -78° alkylate predominately at the α-carbon just as with α,β-unsaturated ketones.[72] The *α-position to the nitrogen* in secondary amines can be activated by N-nitrosation, then metalated and alkylated with electrophilic reagents.[73] Similarly, pyridine-1-oxides may be directly

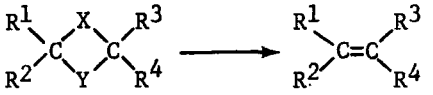


substituted on the α-position(s) by treatment with n-butyllithium in THF at -65° followed by addition of an aldehyde or a ketone.[74]

Chloroheterocycles may be *alkylated* and *alkenylated* by reaction with 2 equivalents of an appropriate Wittig reagent and the new Wittig reagent subjected to hydrolysis to give an alkyl-substituted heterocycle or reacted with carbonyl compounds to elaborate an olefinic side-chain.[75]



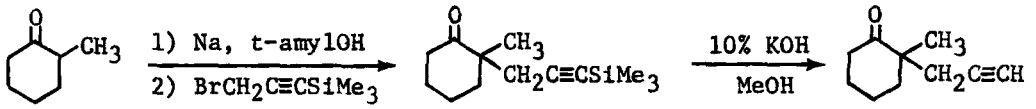
A novel way of preparing olefins has been explored in preliminary experiments. The method uses a *two-fold extrusion*, and the two most promising methods extrude carbon dioxide and sulfur or nitrogen and sulfur.[76]



Methyltriphenoxyphosphonium iodide in hexamethylphosphoramide (HMPA) has been studied as a means of *selectively dehydrating* secondary alcohols without rearrangement. The yields were good, and the more stable alkene predominated.[77] Readily available β-hydroxyalkyl phenylsulfides can be converted to *terminal olefins* by treating the corresponding lithio alkoxide with o-phenylenephosphorochloridate at 0° in THF.[78] A mechanistically similar reaction for the preparation of terminal olefins from non-enoliz-

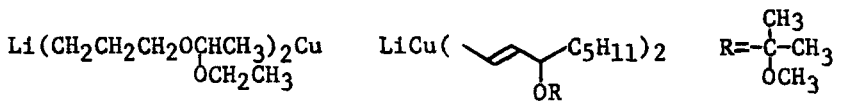
able ketones has also been described.[79]

Acetylenes - Aldehydes can be converted to *terminal acetylenes* in a *one-carbon homologation* reaction by using a modified Wittig reagent prepared from triphenylphosphine and CBr_4.[80] The readily prepared 3-bromo-1-trimethylsilyl-1-propyne is useful for the *propargylation of ketones* with the advantage that the initial trimethylsilylated product can be easily separated from the starting material by distillation.[81]

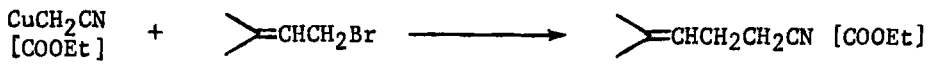


Organocopper Reagents - A review describing the vast potential uses of organocopper reagents in organic synthesis has appeared,[82] and a review of organocopper *conjugate additions* has been published.[83] Many new organocopper reagents have been reported in 1972, and the following examples illustrate advances in the field.

Use of mixed cuprate reagents of the type R_tR_rCuLi, where R_t is the group to be selectively transferred and R_r is the residual group lost during work-up, saves valuable R_t groups and increases the efficiency of the reaction.[84] Oxygen functionalized organocopper reagents of the types shown below have been successfully used in conjugate additions.[85,86,87,88] Vinyl copper reagents have also been reported.[89] *Cyanomethyl copper*[90]



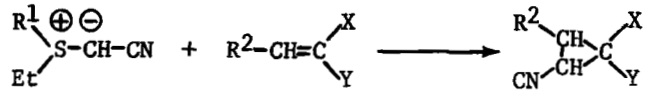
and *carboethoxymethyl copper*[91] react with allylic halides to give, respectively, γ,δ-*unsaturated nitriles* and γ,δ-*unsaturated esters* in good yields. Organocopper reagents have also been used to prepare *aryl*



acetylenes.[92]

Cyclopropane Formation - A zinc-silver couple has been found to be superior to zinc-copper couple in many *Simmons-Smith* reactions of olefins with methylene iodide. Yields are often better, reaction times reduced, and only a slight excess of reagent is necessary to complete the reaction.[93]

α,β-Unsaturated esters can be converted efficiently to *gem-dimethylcyclopropanes* with triphenylphosphonium isopropylide [$Ph_3P{=}C(CH_3)_2$].[94] Cyclopropane derivatives are also formed by reacting α,β-unsaturated nitriles, ketones, and esters with a new, easily prepared cyanosulfonium methylide.[95] A review describes phenyl(trihalomethyl)mercury compounds



as versatile dihalocarbene precursors.[96]

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Chapter 32 - Quantitative Structure-Activity Relationships

W. J. Dunn, III, College of Pharmacy
University of Illinois, Chicago, Illinois

Introduction - In attempting to formalize, in numerical terms, how changes in chemical structure within a series of closely related biologically active agents effect different levels of pharmacologic response, investigators rely mainly on three approaches: 1) the semiemperical linear-free energy related model proposed by Hansch,[1,2,2a,2b] 2) the de novo model proposed by Free and Wilson[3], and 3) quantum mechanically based methods.[4] From surveying the literature of the period since this area was last reviewed[4] it is obvious that the degree of sophistication in applications has increased. Correlations of structure with activity are being determined for much more complex systems, and more laboratories are directing their attention to these approaches.

Utilizing the Hansch approach one attempts to relate a change in level of biological activity with a change in the physical and chemical properties of the series within the framework of equation 1. A_i is the activity of the ith member of the series and can be in terms of a standard or relative biological response.[5] For optimum information and comparative purposes the standard response (A_i = the reciprocal of the molar concentration required to elicit a predetermined response, ED_{50}, I_{50}, etc.) is preferred, but the use of a relative biological response as the dependent variable in structure-function analyses can be useful. The advantages and disadvantages of the form of the activity term are discussed by Tute.[5]

$$\log A_i = -k_1 \log^2 P_i + k_2 \log P_i + k_3 \qquad 1)$$

The term, P_i, of equation 1 is the partition coefficient of the drug between the nonpolar biophase of the biological system and its aqueous phase, and accounts for the lipophilic character of the drug.[6] The k's are constants determined by regression analysis. If activity is a function of the steric and electronic nature of the drug's substituents these are assumed to be included in the term k_3 which can be factored according to equation 2. E_s and σ are the well-known Taft-Hammett constants.

$$k_3 = f(E_s, \sigma, \text{etc.}) \qquad 2)$$

The model derived by Free and Wilson[3] is given in equation 3 where A_i is the activity of the ith member, μ is the overall average activity

$$A_i = \mu + \Sigma a_{j,p} \qquad 3)$$

or contribution to activity from the constant part of the molecule, and $a_{j,p}$ is the contribution from the jth substituent in position p. This

technique, in essence, ranks the substituents in each position according to their contribution to activity. It has been proposed that the Hansch and Free-Wilson approaches, in some cases, can be interrelated.[7]

Quantum mechanical approaches to quantitative structure-activity relationships have their roots in receptor theory. The receptor for a specific drug is thought to have sites through which it can interact with the drug which, for optimum response, must be in a conformation that will allow its heteroatoms to interact with the sites of the receptor.[8] By determining the intramolecular distances between heteroatoms in the preferred conformation of agents which are known to interact with a receptor responsible for a specific response, new drugs can be designed with the appropriate distances fixed between critical atoms.

The application, in a quantitative sense, of the approaches mentioned requires some sophistication in computer techniques and information handling. Recognizing this and in an attempt to accelerate the acquisition of new pharmacological knowledge, the Chemical/Biological Information-Handling Program of NIH is developing computer-based research tools for investigators in this area. One such tool is the PROPHET system,[9] which employs a dedicated, time-sharing computer available via telephone lines. The system, to be made available to selected users in 1973, offers a variety of hardware/software options which should facilitate work in the determination of structure-activity relationships.

Linear Free Energy Models - By far the most extensively used method for determining quantitative structure-activity relationships is the model proposed by Hansch.[1] This model is an extension of the work of Meyer[10] and Overton[11] who showed that narcotic potency is a linear function of lipophilic character according to equation 4. An extensive compilation of structure-activity relationships of this type have been published[12] with 1-octanol/water partition coefficients as a reference for lipophilic

$$\log A_i = a \log P_i + b \qquad 4)$$

character. In this report,[12] a, the slope of the linear-free energy relationship, was considered a measure of the sensitivity of the system to hydrophobic effects. Structure-activity relationships for which $a>0.85$ were classified as hydrophobically sensitive, those with $0.40<a<0.84$ were considered of intermediate hydrophobic sensitivity, and those with $a<0.40$ were considered hydrophobically insensitive. Processes associated with a slope greater than 0.85 are characteristic of drugs interacting with membranes while processes characterized by a slope in the range 0.40 - 0.84 are indicative of drugs interacting with protein.

Equation 4 can be considered a special case of equation 1 which predicts that activity is parabolically related to lipophilic character. This has received additional theoretical justification[13,14] and a recent report by Hansch and Clayton[15] examines the parabolic case for 173 equations in which the addition of the higher order term of equation 1, $-k \log^2 P_i$, is highly significant statistically.

The underlying assumption that makes the model operational is that a nonpolar solvent can be used as a reference system for interaction of drugs with lipoidal biophases. There has been considerable interest in this assumption from the standpoint of which solvent best approximates lipophilicity. 1-Octanol has been most extensively used but there is concern among some workers in this field because of its thermodynamic nonideal nature. On the basis of their more ideal behavior, nonaromatic hydrocarbon solvents have been proposed to best describe apolar interactions[16] and group contributions of the methylene group in a variety of nonpolar solvents have been reported.[17] In cases where it has been possible to actually measure interactions of drugs with biological phases, 1-octanol/water partition coefficients have been a sufficient model for estimating the interaction.[12,18,19] It is most important, in model applications, that the investigator be familiar with the system he uses and know its limitations.

It is assumed that the partition coefficient is an additive-constitutive property of organic molecules[6] according to the relationship $\pi_X = \log P_X - \log P_H$.[6] π_X is the hydrophobic substituent constant and is defined as the difference in the logarithm of the 1-octanol/water partition coefficient of the substituted compound and that of the parent. It is known that there are exceptions to the additivity assumption and the breakdown in some systems has been shown to result from intramolecular electronic and steric interactions.[20,20a]

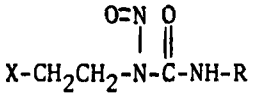
For quantitating some interactions, other than those of an apolar nature, chemical shift values have been explored,[21,22] a method for estimating molar attraction constants has been reported by Cammarata and Yau,[23] and it has been suggested by Deardon and Tomlinson[24] that substituent constants derived from thin layer chromatography be used.

There are pitfalls in using multiple variable regression techniques as a tool for interpreting biological data and some have been discussed.[25,26] Also, guidelines and format for data presentation in order that optimum information may be obtained from structure-function correlations have been suggested.[27]

For those involved in drug design and structural modification with limited access to data handling systems, operational schemes for analog synthesis from a "lead" compound have been proposed by Topliss.[28] It has as its base the Hansch approach and from a practical point of view this is one of the more significant developments during the period covered by this review. This approach is particularly useful in the case where analog synthesis involves several steps and, after synthesis, the biological response for the analog can be obtained rapidly. By using the schemes proposed by Topliss (Topliss Trees) the structure-function relationship can be rationalized in terms of the hydrophobic, steric, and electronic changes. A scheme has been proposed for the case where structural modification occurs on an aromatic nucleus and one for structural modification of an alkyl side chain. By using the appropriate

scheme the most active members of a series can be obtained with a minimum of synthetic effort. Cases have been cited where the schemes could have been utilized[29] and their application to a problem in the design of antineoplastic agents has been reported.[30]

One type of biological response for which few quantitative structure-activity relationships have been determined is that for antineoplastic activity. It is encouraging that applications in this area are being attempted. Structure-cytotoxicity relationships among sesquiterpene lactones have been determined[31] and it was shown that cytotoxicity is largely a function of the lipophilicity of the lactones. Lipophilic character was found to be important in activity against L-1210 in mice for 22 N-nitrosoureas of general structure (I).[32] The quantitative relationship is given in equation 5 where C is the molar concentration required to give a T/C of 175% or an increase in lifespan of 75% for



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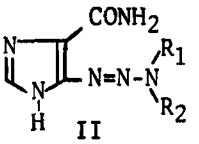


II

treated animals. The optimum lipophilic character, log P_o, obtained from the derivative d(log 1/C)/d(log P) was found to be -0.60 which indicates that activity against L-1210 is greater for N-nitrosoureas with low lipophilic character. Also reported were structure-activity relationships for a series of imidazole carboxamides of structure (II), where R_1 and R_2 are H, alkyl, or alkylaryl.[32] If R_1 and R_2 are both greater than

$$\log 1/C = -0.057(\pm 0.07)\ \log^2 P - 0.069(\pm 0.17) \log P + 4.527(\pm 0.17) \qquad 5)$$

$$N = 22 \qquad R = 0.922 \qquad s = 0.163$$

methyl no activity is obtained. Activity is related to structure in this series by equation 6. The standard response selected for this series was the concentration required to give a T/C value of 150%. For this series log P_o was found to be 1.10 and the negative coefficient for E_s, the Taft-steric constant, was interpreted to mean that bulky groups enhance antitumor activity.

In regard to carcinogenic activity of polycylic aromatic hydrocarbons, it has been reported that these agents induce aryl hydrocarbon hydroxylase (benzopyrene hydroxylase)and inducing potency is a function of hydrophobicity, chemical reactivity, and the ability to participate in

$$\log 1/C = -0.280(\pm 0.14)\ \log^2 P + 0.539(\pm 0.18)\ \log P$$
$$-0.168(\pm 0.15)E_s + 3.430(\pm 0.14) \qquad 6)$$

$$N = 10 \qquad R = 0.969 \qquad s = 0.104$$

charge-transfer interactions.[33] The rate-determining step was proposed to be the formation of a K-region metabolite of the hydrocarbons.[33]

Linear-free energy relationships have appeared dealing with the hydrolysis of p-nitrophenyl esters by α-chymotrypsin,[34] the inhibition of acetylcholinesterase by diethyl phenylphosphates,[35] the inhibition of alcohol dehydrogenase by esters, alcohols, and amides,[36] the inhibition of urease by hydroxamic acids,[37] and the inhibition of phenethanolamine N-methyltransferase by amphetamines.[38,39] In all the cases cited, bonding due to hydrophobic effects was found to be important in the mechanism of activity studied.

Closely related to the enzyme studies are those of microsomal drug metabolism,[40,41] which indicates that hydrophobic effects are important in in vitro and in vivo drug metabolism.

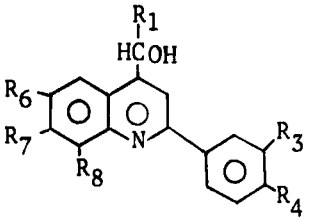
Antibacterial agents have received attention, especially the macrolide antibiotics[42,43] and the structure-activity relationships for the antimicrobial esters of 4-hydroxybenzoic acids have been reported.[44] A survey of the requirements for antifungal activity has appeared.[45] The use of physicochemical parameters and regression analysis in pesticide design has also been surveyed.[46]

Dillingham, Mast, Bass, and Autian[47,47a] have described the toxicological properties of a series of methyl and halogen substituted alcohols, and using both the Hansch and Free-Wilson approaches, Lawrence, Bass, Purcell, and Autian[48] determined the structure-toxicity requirements for esters of acrylic and methacrylic acids in mice. The latter report, which found that the charge on the carbonyl carbon and log P were important in determining the toxic response, is important in view of the usefulness of the agents studied as monomers in the manufacture of polymeric dental supplies. Other investigations using the Hansch approach have appeared which include studies of antihypertensive agents,[49] CNS agents,[50,51,51a] protein binding,[52] general anesthetics,[53] antiinflammatory agents,[54] and bronchodilaters.[54a]

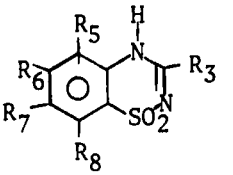
Free-Wilson De Novo Model - The most important information to be gained from a Free-Wilson analysis is the relative rank of substituent group contributions to activity at each position. The methods of application, aspects of interpretation, and statistical requirements for its use are to be found in a report by Craig.[55] Using this approach the substituent contributions to antimalarial activity of a series of sixty-nine 2-phenylquinoline-4-carbinols (III) were assessed by Craig.[56] The most effective substituents were found to be R_1 = 6-methyl-2-piperidyl, R_3 = methoxy, R_4 = iodo, and $R_6 = R_7 = R_8$ = trifluoromethyl. The results significantly support the additivity concept assumed by the Free-Wilson approach. An attempt to analyze the data in terms of the Hansch multiparameter approach was unsuccessful.[56]

The relative contribution of substituents toward the antihypertensive activity for a series of 2H-1,2,4-benzothiadiazine 1,1-dioxides (IV)

were determined by Tinland, Decoret, and Baden.[57] Substituents in position 8 made little contribution to activity while R_3 = Δ^3-cylopentenyl, R_5 = bromo, and R_6 = R_7 = trifluoromethyl made the greatest contributions to activity in the respective positions. The original



III



IV

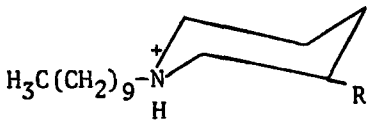
report[49] of the antihypertensive activities were accompanied by a Hansch analysis which showed a marked dependency of activity on the π values of substituents in positions 6 and 7. For the substituents present in positions 6 and 7, the trifluoromethyl group was the most lipophilic[58] with the exception of $\pi_{R_7=I}$ = 1.32 VS $\pi_{R_7=CF_3}$ = 1.22. The Free-Wilson analysis, therefore, corroborates the analysis of Topliss and Yudis.[49]

Quantum Mechanical Approaches - This approach to drug design has been termed quantum pharmacology by Cammarata.[4,4a] It is in its early stages of development and as practical restrictions are being overcome the method is becoming more applicable. Most applications to problems of interest to the pharmacologist and medicinal chemist can be categorized into two general areas: 1) calculating parameters which can be used as indices of chemical and physical properties of pharmacological agents, and 2) determining preferred conformations of medicinal agents.

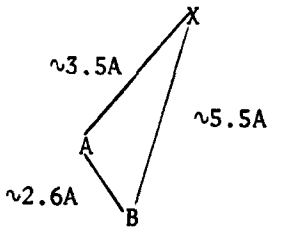
In the former case indices such as superdelocalizability, the energies of the highest occupied molecular orbital (HOMO) or lowest unoccupied molecular orbital (LUMO), or charge densities at particular sites are calculated. These indices are then used as independent variables in conjunction with regression techniques in order to determine changes in activity as a function of changes in the electronic nature of medicinal agents. It has been pointed out by Andrews[59] that care must be exercised in using indices obtained by various quantum mechanical methods and he has suggested that CNDO/2 and ab initio methods are probably adequate for predicting qualitative charge variations in atoms separated by as many as three bonds from a molecular modification while EHT methods of calculation of charge variations may be inadequate.

Using this approach Millner and Purcell[60] determined the change in net charge densities on quaternary nitrogen, carbonyl carbon, and carbonyl oxygen in a series of 9 butyrylcholinesterase inhibitors of structure V where R = $NHCOR_1$, or $CONR_2R_3$. There was found to be no dependence in inhibitory potency on the charge densities calculated. The results were in agreement with earlier findings[61] that inhibitory potency was dependent upon the lipophilic nature of R.

Applications of the latter type are exemplified in the receptor mapping technique of Kier[8] who has determined the preferred conformations of sweetening agents.[62] Schallenberger and Acree[63] have proposed a receptor which will accommodate sweet molecules. This model consists of an electronegative atom B and a polarizable A-H group. The A-B distance is found to be 2.5 - 4.0 A in sweet molecules. From calculations of preferred conformations of known sweetening agents a third structural feature capable of charge transfer or dispersion bonding has been proposed[62] and its position relative to the B and A-H centers is given below. Because of interest in artificial sweeteners this proposal will surely receive attention.



V



A recent evaluation of the receptor mapping technique failed to yield an agent which would block oxotremorine induced tremors.[64]

Other molecular systems of biological importance on which computational studies were performed are acetylcholine and its agonists, muscarine and nicotine[65] prostaglandin E_1,[66] gastrin tetrapeptide,[67] and phenethylamines.[68] An evaluation of interatomic distances calculated from the preferred conformations of 9 phenethylamines[68] suggested possible features of their receptor and further studies[69] of sympatholytic agents containing the C_6H_5-X-$CH_2\overset{+}{N}H$< grouping (X=O-CH_2, -$NHCH_2$, -O-) suggest that only those drugs with X = -O-CH_2 will exist in a conformation similar to the phenethylamines.[69]

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Chapter 33. Metal Carbonyls as Reagents and Intermediates for Organic Synthesis

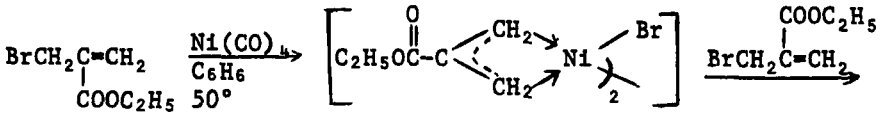
Howard Alper, Department of Chemistry,
State University of New York at Binghamton, Binghamton, New York

Introduction - The past twenty years have witnessed an increased awareness of the importance of transition metal organic compounds in chemical research.[1-3] The major part of this research has been concerned with the utilization of these compounds as stoichiometric reagents for organic synthesis. This chapter reviews the recent organic chemistry of transition metal carbonyls, a class of organometallics important as reagents and as intermediates for organic synthesis. The outstanding chapter by Calderazzo, Ercoli, and Natta in Wender and Pino's book on "Organic Syntheses via Metal Carbonyls" is recommended as an introduction to the structure, synthesis, and chemical reactivity of metal carbonyls.[4] Several reviews dealing in whole, or in part, with metal carbonyls as reagents exist in the literature.[5-8]

Metal Carbonyls as Reagents

I. Coupling and Dehalogenation Reactions of Organic Halogen Compounds - cis-Hydridotetracarbonyl(triphenylphosphine)manganese can convert alkyl, allyl, and benzyl halides to hydrocarbons in good yields. Allylic bromides gave the isomerized as well as the expected products.[9]

It has been known for some time that allylic bromides react with nickel tetracarbonyl [$Ni(CO)_4$] to form π-allylnickel bromide complexes (e.g., 1), which, in turn, can be coupled with the same or with a different bromide (or iodide) to give simple or functionalized alkenes. This reaction sequence has recently been applied to the synthesis of monoterpene derivatives, including geranyl acetate.[10] The π-allylnickel halide intermediate need not be isolated in certain instances, e.g., treatment of ethyl α-bromomethylacrylate with $Ni(CO)_4$ gave 2 in 70% yield.[10] π-Allylnickel bromide complexes have been used in two dif-



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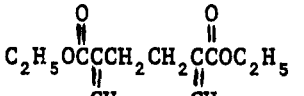

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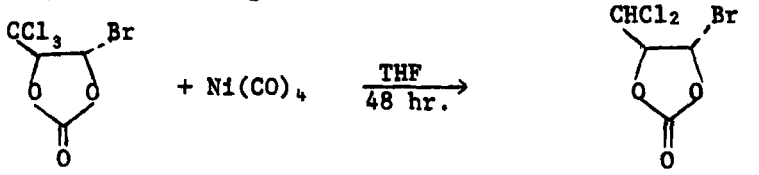
ferent preparations of Coenzyme Q_1.[11,12] An example of intramolecular coupling of an appropriate allylic dibromide by $Ni(CO)_4$ in N-methylpyrrolidone to form a macrocyclic lactone has been communicated and shows promise as a new approach to these important compounds.[13]

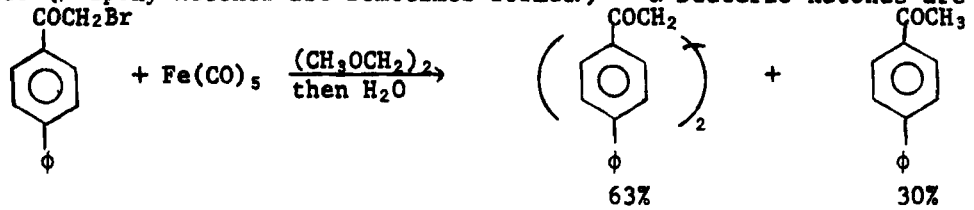
Dicobalt octacarbonyl [$Co_2(CO)_8$] is a useful reagent for converting geminal and vicinal-dihalides to alkenes.[14] The use of iron pentacarbonyl [$Fe(CO)_5$],[15] for effecting the same transformations had been described earlier.

Mono or dihalogenomethyls can be obtained when polyhalogenomethyls are treated with $Ni(CO)_4$ in tetrahydrofuran.[16] The reaction can occur in the presence of monohalides (3 → 4) and has been used in a new, stereoselective synthesis of sugar derivatives.[16,17]



3 4, 62%

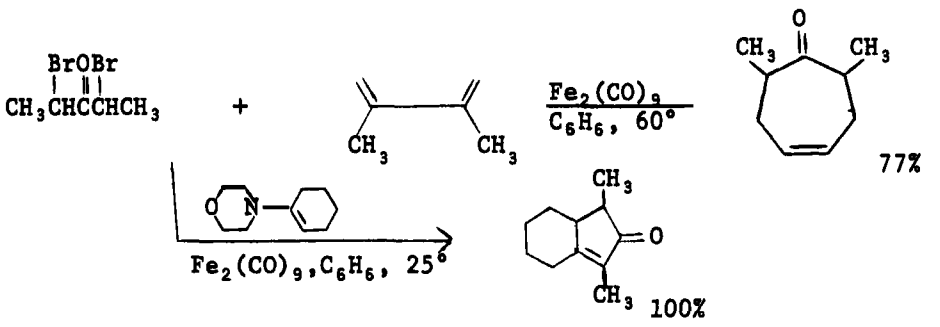
Reaction of $Fe(CO)_5$ with various aryl and alkyl α-bromo (1°, 2°, or 3°) ketones in hot 1,2-dimethoxyethane, and subsequent aqueous work-up, generally gives the coupled 1,4-diketones and reduced monoketones in good yields (β-epoxy ketones are sometimes formed.)[18] α-Deuterio ketones are



63% 30%

formed when the reaction is worked up using D_2O instead of H_2O. Unsymmetrical 1,4-diketones can be prepared by first isolating the intermediate organoiron bromide [from α-bromo ketone and diiron enneacarbonyl ($Fe_2(CO)_9$) at room temperature] and then reacting the latter with a different α-bromo ketone in the presence of $Fe(CO)_5$. The mechanism of this reaction is apparently similar to that of other useful coupling reactions effected by $Fe(CO)_5$ including the synthesis of alkenes from vic or gem-dihalides,[15] and the preparation of thiolsulfonate esters from sulfonyl chlorides.[19] Two convenient disulfide syntheses, one from sulfonyl chlorides and group VI metal carbonyls [$M(CO)_6$, M = Cr, Mo, W],[20] and the other from sulfenyl halides and $Cr(CO)_6$, $Fe(CO)_5$, or $Ni(CO)_4$,[21] may also occur by a pathway similar to that for the α-bromo ketone-$Fe(CO)_5$ reaction.

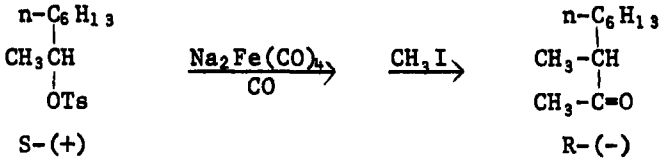
The reaction of α,α'-dibromo ketones with $Fe_2(CO)_9$ in the presence of 1,3-dienes[22] or enamines[23] represents an elegant approach to 4-cycloheptenones and 2-cyclopentenones, respectively. A mechanism, initially similar to that described for the α-bromo ketone-$Fe(CO)_5$ reaction, has been proposed for these cyclization reactions.[24]



Biphthalidylidene has been isolated in 23% yield from the interesting reaction of phthaloyl dichloride with sodium tetracarbonylferrate (-II) [$Na_2Fe(CO)_4$].[25]

II. Carbonylation - In 1970, Cooke[26] reported that primary (but not benzylic) bromides and a secondary bromide could be converted into aldehydes (50-99%) using $Na_2Fe(CO)_4$ and triphenylphosphine in tetrahydrofuran. Complementing this reaction is the preparation of aromatic aldehydes in 24-65% yields when $Fe(CO)_5$ was rapidly added to a dilute ether solution of an aryl lithium at -60° (followed by acidic work-up).[27] The intermediate acyl tetracarbonylferrate (in situ) of the last reaction can also be generated by reaction of an acid chloride with $Na_2Fe(CO)_4$ in tetrahydrofuran. Acidification gave aldehydes (aliphatic and aromatic) in good yields.[28]

Of greater potential than the $Na_2Fe(CO)_4$ mediated conversion of bromides to aldehydes is the synthesis of ketones by carbonylation of bromides, iodides, or tosylates using the same reagent.[29] This stereospecific reaction can be executed in a number of ways including treatment of the dianion successively with two different halides to form unsymmetrical ketones. The latter can also be produced by initial generation of an alkyl tetracarbonylferrate (0) [from $Na_2Fe(CO)_4$ and halide or tosylate],[30,31] carbonylation to the acyl tetracarbonylferrate, followed by treatment with a different halide. The last step of the reaction sequence was previously reported by other workers.[32]



High yields of ketones were obtained by carbonylation of aryl mercuric chlorides or bromides using $Co_2(CO)_8$ in tetrahydrofuran[33] or $Ni(CO)_4$ in N,N-dimethylformamide.[34] These reactions can be applied with moderate success to alkyl mercuric halides.

Olefins can undergo stoichiometric hydroformylation with cobalt tetracarbonyl hydride in non-polar solvents to give aldehydes and $Co_2(CO)_8$.[35,36] Markovnikov addition to unsymmetrical alkenes is generally the predominant process, resulting in the formation of branched chain aldehydes. The straight chain aldehyde is occasionally favored when the reaction is effected under one atmosphere of carbon monoxide. The catalytic hydroformylation reaction has been investigated in considerable detail.[37,38]

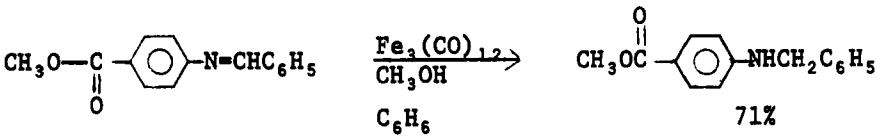
Irradiation of equimolar quantities of vinyl cyclopropanes and $Fe(CO)_5$ [or $Fe_2(CO)_9$] in benzene or hexane solution results in a novel 1,5-carbonyl insertion to give 2-cyclohexenones.[39] Another carbonylation-cyclization process takes place when a mixture of diphenylketene and diaryl acetylene is reacted with $Fe(CO)_5$ or $Ni(CO)_4$.[40] 3-Cyclopentene-1,2-diones or 4-cyclopentene-1,3-diones were the major reaction products, subject to the nature of the metal carbonyl reagent. Similarly, alkynes react with benzyl bromide or β-bromostyrene and $Ni(CO)_4$ to form dimers of γ-but-2-enolactones in 30-65% yield.[41]

Iron pentacarbonyl can effect carbonyl insertion by reaction with aliphatic and cycloalkyl nitro compounds in dry diglyme at 120-132° to give formamides and ureas in modest yields.[42] The same reagent can convert non-aromatic nitrosamines to tetrasubstituted ureas (and formamides as by-products).[43,44]

III. Reduction - The double bond of α,β-unsaturated carbonyls and nitriles can be selectively hydrogenated by hydridoiron carbonyl complexes, which were generated *in situ* by reaction of $Fe(CO)_5$ with a small quantity of base in moist solvents.[45] The stereochemistry of the reaction varies.

Iron pentacarbonyl is a useful reagent for deoxygenating amine oxides, azoxy benzenes, nitrones,[43] and sulfoxides.[46] It is also important for effecting reductive coupling of aromatic nitro compounds to azo, azoxy compounds and/or amines, the nature of the product(s) subject to reagent concentration, and the substitution pattern in the benzene ring. Simple aromatic nitroso compounds react with $Fe(CO)_5$ to form azobenzenes while the latter, amines and/or benzoquinones are possible products when a nitrosophenol was the organic reactant.[43] Treatment of aromatic nitrosamines with $Fe(CO)_5$ or $Mo(CO)_6$ results in the formation of secondary amines in high yields [$Cr(CO)_6$ and $W(CO)_6$ were less effective reagents].[43,44]

The hydridoundecacarbonyltriferrate anion $[HFe_3(CO)_{11}^-]$, generated *in situ* from triiron dodecacarbonyl $[Fe_3(CO)_{12}]$ and methanol, is a promising new hydride reagent for organic synthesis. It specifically reduces aromatic nitro compounds to amines in the presence of functionalities such as NH_2, COOR, OH, ketone, halide, or amide.[47] A carbon-nitrogen double bond of a heterocyclic diazine or of Schiff bases con be easily reduced using this reagent.[48]



IV. Miscellaneous - Iron carbonyls react with benzylidene and alkylidene phosphoranes (phosphorus ylids) in refluxing tetrahydrofuran to give stilbenes in low to moderate yields.[49] 1,4-Diketones were isolated from reactions of $Fe(CO)_5$ with phenacylidene phosphorus, nitrogen, or sulfur ylids. Olefins can also be obtained from $Fe(CO)_5$ and thionacarbonates.[50] Yields range from 10.5-79.1% and the reaction displays little stereospecificity.

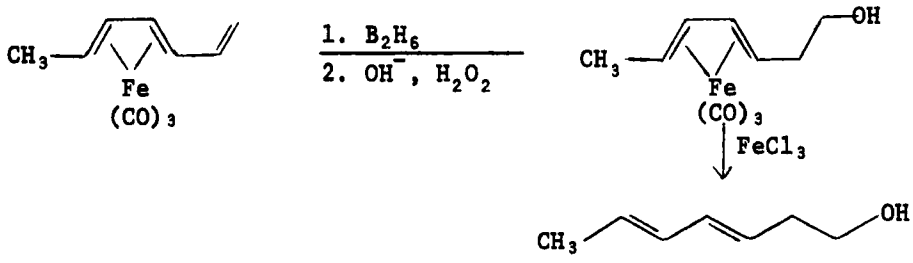
Maleic anhydride-iron tetracarbonyl is a useful reagent for protein modification.[51]

Metal Carbonyls as Intermediates for Organic Syntheses

I. Olefin, Alkyne, and Arene Metal Carbonyl Complexes - A potentially important new route to optically active amino acids has been described.[52] The iron tetracarbonyl complex of L-(+)ethyl α-methylbenzyliminoglyoxalate, obtained by treatment of the ligand Schiff base with $Fe_2(CO)_9$, was converted to D-(+)-phenylalanine (77% optical purity, 53% yield) by reaction with benzyl bromide, and subsequent hydrogenation.

Stereospecific deoxygenation of epoxides to olefins [retention of configuration] can be accomplished by use of sodium (cyclopentadienyl) dicarbonyl ferrate as the reagent.[53] Sodium iodide was used to liberate the olefin from the intermediate olefin(cyclopentadienyl)iron dicarbonyl cation.

Transoid steroid dienes can be isomerized to the thermodynamically less stable homoannular cisoid isomers via diene-iron tricarbonyl complexes.[54] Reaction of the pentadienyl iron tricarbonyl cation derived from cholesta-1,3-diene or cholesta-2,4-diene, with nucleophiles followed by oxidative cleavage gives cholesta-1,3,5-triene.[55] Hydroboration of the uncomplexed double bond of acyclic or cyclic triene-iron tricarbonyl complexes and subsequent oxidation gives good yields of unsaturated alcohols difficult to synthesize by other routes.[56]



A simple preparation of cyclobutadienopleiadene, and a dimethylene cyclobutene, from cyclobutadiene-iron tricarbonyl has been communicated.[57] Also reported is the conversion of cycloheptatrienes to azulenes via heptafulvene-chromium tricarbonyl.[58]

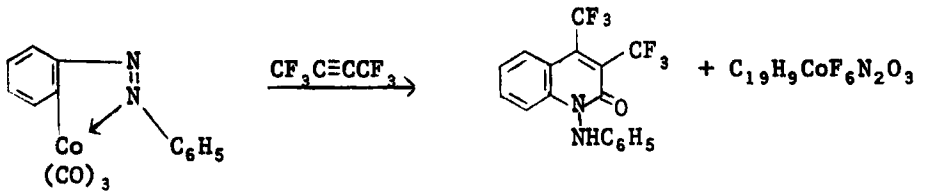
Phenylmethylidynetricobalt nonacrbonyls [$ArCCo_3(CO)_9$] undergo Friedel-Crafts acylation at the para-position.[59] Oxidation with ceric ion gives 4-keto benzoic acids. New chemistry of other alkylidynetricobalt nonacarbonyls suggests the development of reactions useful for organic synthesis.[60,61] Acetylenes react with $Co_2(CO)_8$ to form acetylene dicobalt hexacarbonyl complexes. Complexes derived from diarylacetylenes (e.g., diphenylacetylene) also undergo Friedel-Crafts acylation at the para-position, and on oxidative cleavage, give the substituted acetylenes in reasonable yields.[59] Of particular interest in the area of oral contraceptives is the use of the dicobalt hexacarbonyl moiety as a protecting group for an alkyne function in a steroid while effecting various manipulations on a double bond present in the same molecule.[62]

Treatment of readily prepared arene mangenese tricarbonyl cations with cyanide ion, followed by oxidative cleavage, results in the conversion of arenes to nitriles in good yield.[63]

II. Carbonyl Carbene Complexes - Group VI metal carbonyl carbene complexes, prepared from $M(CO)_6$ [M = Cr, Mo, W] and organolithium reagents,[64,65] react with α,β-unsaturated esters[66] and ethers[67] in stereospecific fashion to give cyclopropane derivatives (along with other products) and with alkylidenetriphenylphosphoranes in ether at room temperature to form ethers in high yields (Wittig type reaction).[68] A novel synthesis of 2-pyrones has been achieved by initial reaction of cyclopropylidene chromium or molybdenum pentacarbonyl carbene complexes with pyridinium ylids to form pyran-2-ylidene carbene carbonyl complexes. The latter were converted to 2-pyrones by treatment with lead tetraacetate.[69] The anion, formed by removal of a proton on a carbon atom adjacent to the metal-carbon double bond of a carbonyl carbene complex, can undergo condensation with aldehydes or methylation with methyl fluorosulfonate.[70]

Hydrogen transfer from nitrogen to carbon has been observed in the reaction of secondary amino(phenyl)carbene chromium pentacarbonyl complexes with excess pyridine. Schiff bases were obtained in good yield by this route.[71]

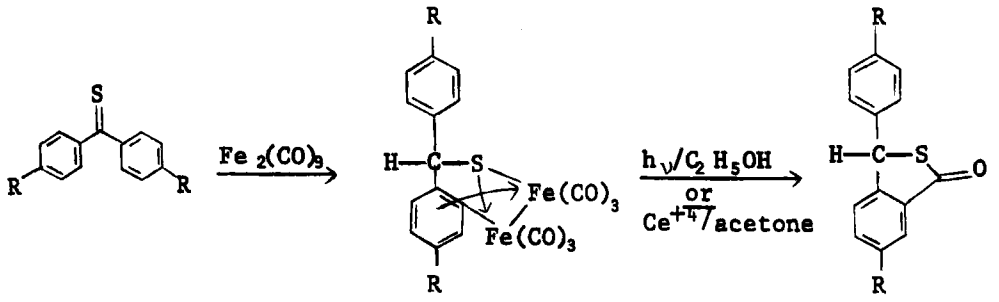
III. Intramolecular Aromatic Substitution via Metal Carbonyl Complexes - A relatively new class of aromatic substitution reactions are known whereby a metal atom replaces a hydrogen attached to an ortho-position of a benzene ring.[72] The nitrogen-donor cobalt tricarbonyl complex 5 [from azobenzene and $Co_2(CO)_8$] reacts with hexafluoro-2-butyne to give the 2-quinolone (6) in good yield and a cobalt complex 7.[73] Should this reaction prove to be a general one, it would constitute a simple route to 2-quinolones. Carbonylation of 5 in methanol gave 2-



5 6, 65% 7, 25%

carbomethoxyhydrazobenzene in low yield.[74] N-Phenylphthalimidine was obtained in 48% yield by ferric chloride treatment of the ortho-metallated complex derived from N-benzylideneaniline and $Fe_2(CO)_9$.[75] A similar conversion of the anil of 2-naphthaldehyde to 1-oxo-2-phenylbenz-[f]isoindoline has been described.[76]

Thiobenzophenones react with $Fe_2(CO)_9$ in benzene at room temperature to give sulfur-donor ligand ortho-metallated complexes (of type 8) in good-excellent yields.[77] Irradiation of these air-stable complexes in ethanol or treatment with ceric ion gives thiolactones (9) in 58-81% yield, thus providing a simple (two-step) entry into the isobenzothiophene ring system.[78]



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Chapter 34. Biopharmaceutics and Pharmacokinetics

Ho-Leung Fung, School of Pharmacy
State University of New York at Buffalo

BIOPHARMACEUTICS

Bioavailability - The subject of bioavailability has aroused much discussion among governmental, industrial and various professional groups. A conference for the purpose of discussion on the capabilities of bioavailability testing and control, as well as the perceived need for public standards, was held late in 1971, and the proceedings are now available in a text[1] entitled, "Bioavailability of Drugs." The Food and Drug Administration has recently outlined proposed regulations[2] which define bioavailability, methods for establishing bioavailability of a drug and general requirements for establishing bioavailability. The Canadian Health Protection Branch of the Department of National Health and Welfare has elected to consider as acceptable a bioavailability of 80% or more of a reference standard[3]. This blanket criterion for all drugs was rightly challenged[4] on grounds of individual drug dose-response relationships and the possibility of capacity limited metabolism. The inappropriateness of classical hypothesis testing techniques when applied to comparative bioavailability trials was pointed out.[5] An alternate statistical treatment utilizing confindence intervals was then proposed. Chiou[6] and Ritschel[7] both pointed out the importance of sufficient data collection beyond the absorptive phase when areas under the curve are to be used in bioavailability determinations.

The original work on the bioavailability of digoxin tablets by Lindenbaum et al.[8] stimulated a number of studies on the subject. In that report "Lanoxin" tablets were shown to be more bioavailable than other brands. On the other hand, Shaw et al.[9] showed little bioavailability difference among these brands. These conflicting results could arise from the fact that the two studies utilized different formulations of "Lanoxin" which had significantly different dissolution characteristics.[10] Huffman and Azarnoff[11] showed that "Lanoxin" tablets are 75% bioavailable when compared with an oral solution of digoxin which is completely absorbed. While little bioavailability differences exist between three tablet brands of diazepam [12], and between oral vs. intravenous administration of doxycycline hyclate[13], significant bioavailability differences among commercial brands were found for tetracycline[14], sulfamethizole[15] and ampicillin[16]. In the last two studies, in vivo differences could not be correlated with dissolution data obtained using the U.S.P. or other methods. The biological availability of intact alprenolol[17] and propranolol[18]were both affected by dose dependent first pass elimination. There exists a threshold dose level (approximately 30 mg for both drugs) below which the drugs are completely unavailable to the general circulation because of complete biotransformation during first pass through the gastrointestinal tract and the liver. Extensive absorption was observed for the ionic methylene blue in man, although not in the dog[19]. Pfeffer and

Schor[20] showed that disappearance of isotropine methylbromide in an in vivo intestinal loop of the rat does not reflect circulatory uptake of the drug. A significant portion of the drug appears to be reversibly bound to the intestinal wall and is not available to the general circulation. Significant hydrolysis in the gut was shown for propantheline bromide[21]; the oral absorption of intact drug was estimated to be less than 50%. Similarly, only 68% of an oral dose of aspirin reached the peripheral circulation intact[22] because of hydrolytic degradation to salicylic acid.

Effects of Adjuvants on Absorption - Vaidhyanathan[23] presented theoretical considerations which allowed a priori prediction of whether complex formation will lead to enhancement or reduction of the transport rate of a drug across biological membranes. Increase in steroid absorption by complexation with dialkylpropionamides was demonstrated using a modified in situ rat intestine technique which allowed for maintenance of a constant concentration of the complexing agent[24]. The absorption of a variety of non-steroidal drugs was not affected by N,N-di-n-propylpropionamide, even though complexation between these drugs and the amide in an organic solvent, as well as enhanced transfer through an artificial lipoid barrier, could be demonstrated[25]. Stupak and Bates[26] showed that the fraction of the oral dose absorbed in the rat was three times greater with the 1:5 reserpine-polyvinylpyrrolidone coprecipitate than with either the pure drug or the 1:5 reserpine-PVP physical mixture. The in vivo absorption data correlated well with in vitro dissolution characteristics. Naproxan, a carboxylic acid, was completely orally available. Surprisingly, tablet formulations with an extraordinarily long disintegration time or with an alkalinizing agent did not alter absorption characteristics[27]. Increase in in situ absorption of quinine and chlorpheniramine with anions[28] cannot be solely attributed to the partition behavior and surface activity of ion-pair complexes.

Adjuvants may have an effect on the tonicity of drug solutions, which in turn cause net water flux accompanying drug absorption. Ochsenfahrt and Winne[29] assumed that different osmolality did not change the permeability coefficient and surface area and suggested that water net flux influenced absorption of aminopyrine and antipyrine by interaction between water and drug molecules within the lipid part of the cell membrane (solvent drag). Kojima et al.[30] showed that no apparent difference in the permeability of the membrane to the hypotonic and isotonic sulfaethidole solutions was observed if suitable corrections for volume and surface area changes are made. The apparent permeability of the membrane to hypertonic solution was, however, significantly decreased.

Effect of Physical and Chemical Derivatization on Availability - The question of aspirin polymorphism has not yet been resolved; conflicting evidence for[31] and against[32] the existence of the more absorbable Form II has been presented. The methanesulfonate and chloride salts of pralidoxime, when given on an equimolar basis, gave comparable plasma levels[33]. Oral activity was conferred on carbenicillin when the 5-position is modified by forming the indanyl ester, which is absorbed and subsequently cleaved by serum and tissue esterases to yield the parent antibiotic[34].

2,2'-Anhydro-ara-C[35] and 2,2'-Anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine[36] possess much higher oral activities than their parent compounds against intraperitoneally and intracerebrally inoculated mouse leukemia, possibly due to decreased biotransformation on first pass. Δ^9-Tetrahydrocannabinol, which is resinous and insoluble in water and, therefore, difficult to study pharmacologically, can be converted to bifunctional esters which are water soluble[37].

Drug Interactions Affecting Oral Absorption - The absorption of riboflavin-5'-phosphate was enhanced when co-administered with the highly viscous sodium alginate, which allowed for prolonged retention of the vitamin at the specialized absorption sites in the small intestine[38]. This same mechanism can be invoked to rationalize increased absorption of riboflavin when co-administered with propantheline[39] and to explain differences in riboflavin absorption in hypothyroid, euthyroid and hyperthyroid children[40]. Similar, though not as dramatic, results[41] with respect to the rate of absorption of pivampicillin and tetracycline in man were observed after pretreatment with metoclopramide, which stimulates peristalsis and atropine, which has the opposite effect. Increased absorption of aminopyrine when co-administered with barbital is rationalized by neutralization of the inhibitory effect of aminopyrine on gastric emptying[42]. Increase in pseudoephedrine absorption by concurrent administration of aluminum hydroxide gel[43] was ascribed to an increase in the amount of the more absorbable non-ionized form, although pKa considerations indicate very little increase in the fraction of non-ionized form. Different dosage forms of activated charcoal were found to significantly inhibit the absorption of aspirin[44]. Cholestyramine was found to significantly inhibit absorption of sodium fusidate in the rat[45]; prolonged administration of the anionic exchange resin to rats also resulted in significantly decreased serum iron, tissue nonheme iron stores and serum vitamin B_{12} levels associated with decreased hematocrit and hemoglobin levels[46]. It was possible to avoid interference of oral tetracycline absorption by iron if the latter is administered not less than three hours before or two hours after tetracycline[47]. Diphenylhydrantoin was found to inhibit absorption of folic acid[48]; this may explain, at least in part, the association of diphenylhydrantoin therapy and folate deficiency.

Absorption Through Other Routes of Administration - Buccal absorption data of substituted phenylacetic and toluic acids have been analyzed quantitatively by using a two-phase compartmental diffusion model[49]. The polarity of the lipoidal buccal membrane appeared to resemble that of isobutanol. Solid flakes of pilocarpine alginate deposited in the cul-de-sac of the rabbit eye gave prolonged release characteristics when compared with a solution containing the same amount of the drug alginate[50]. Absorption of a number of drugs from the rat lung is shown to be much more rapid than from the gastro-intestinal tract. Lung absorption data could be explained in terms of diffusion of solutes through at least three different populations of aqueous membrane pores of various diameters[51]. The absorption of disodium chromoglycate through the lung is rapid, but most of the inhaled dose is swallowed and is not available for absorption from the gastro-intestinal tract. About half of the absorbed drug is excreted in the bile. Similarly, a majority of an aerosol dose of salbutamol is swallowed[52].

However, improvement of lung function after aerosol administered salbutamol was observed before there were measurable plasma levels of the drug. Thus, it appeared that the aerosol acts topically and physiologic effects cannot be correlated with blood levels. The methods of measuring and factors affecting percutaneous absorption were reviewed[53]. The pharmacokinetics of several ephedrine analogs after percutaneous and oral administrations were compared[54]. Absorption through the percutaneous route is always slower and did not correlate with the partition coefficient of the drug. Intravenously administered emulsions of barbiturates were shown to give a more prolonged duration of sleep when compared to aqueous IV solutions of their sodium salts[55]. The onset of action was immediate for both preparation forms of the ultra-short acting barbiturates, but a slight prolongation was noticed with emulsions of the short acting barbituric acids.

PHARMACOKINETICS

Correlation with Pharmacological Effects - The use of pharmacokinetic parameters to describe time dependent clinical or pharmacological effects is a major achievement of the discipline of pharmacokinetics. Reviews on the kinetics of reversible pharmacologic effects in intact animals including man[56] and in pediatric pharmacology[57] have been presented. Utilizing engineering optimization techniques and computer simulation, Smolen et al.[58] demonstrated the use of time-optimal drug input systems which can theoretically produce optimum response in the desired pharmacological effect without exceeding toxic response intensities in other simultaneously occurring pharmacological effects. Smolen et al.[59] also derived mathematical relationships between dose, effect, time and biophasic drug levels, which in principle would allow for determination of biophasic drug levels from observed intensities of pharmacological responses. A three-compartmental linear model, in which the site of action resides in the central compartment, has been constructed for d-tubocurarine by Gibaldi et al.[60] This model successfully predicts the pharmacologic effects on various dosage regimens and can be used to rationalize the dose-dependent duration of the neuromuscular blocking effect of d-tubocurarine in patients with renal failure[61]. Using tubocurarine as an example, these authors showed that the rate of decline of pharmacologic effects of drugs with multicompartment characteristics decreases with increases in dose. This is in contrast to the dose independent relationship in cases when the drug is eliminated monoexponentially[62]. Jusko[63] delineated the dose-effect relationships of teratogens through extension of the deterministic approach to the bimolecular and irreversible interaction of chemicals with receptors which he previously applied to antineoplastic drugs. On the basis of the dose-effect curves, teratogens are classified into two categories, viz.: (i) those which have no minimum embryopathic dose and (ii) those which require a threshold number of drug-receptor complexes before teratogenic effects take place.

Mathematical Properties of Pharmacokinetic Models and Parameters - Through the use of general input and disposition functions, the method of partial fractions for solving Laplace transforms, and a multiple-dosing function, Benet[64] derived extremely useful general mathematical treatments which

greatly simplified derivation of equations for any linear mammillary compartment model with any first-or zero-order input process. Jusko and Gibaldi[65] examined the mathematical behavior of various pharmacokinetic parameters of the two-compartment open model upon a change of magnitude in the elimination rate constant (k_{el}). The theoretical predictions are consistent with experimental data of (i) reduced benzylpenicillin renal clearance by probenecid[65] and (ii) several drugs in patients with renal failure[66]. Ho et al.[67] derived the mathematics of a physical model for the absorption of drugs applicable to situations in which the diffusional flux of the drug might be influenced by the bulk fluid flow and surface pH. Notari et al.[68] pointed out that the absorption rate constant calculated from appearance of drug in the blood was an apparent constant which was the sum of all individual rate constants for simultaneous first-order loss of drug from the absorption site. Using the "first pass" pharmacokinetic models, Gibaldi and Feldman[69] examined the influence of route of administration on drug and metabolite levels in the tissue, the percent of dose excreted in the urine as unchanged drug and the composition of total metabolites in the urine. Pharmacokinetic properties of a number of drugs can be predicted by these models. By using area analysis following intravenous administration, blood flow information and clearnace concepts, Rowland[70] derived a mathematical relationship which allows an estimate of the availability of a completely absorbed, orally administered drug, which, however, is subjected to "first pass" metabolism.

Pharmacokinetic Data - During the past year, a large number of papers were published which provide useful pharmacokinetic data for various drugs in man and other animals. However, it is beyond the scope of this review to offer a comprehensive documentation of these papers, and many studies which only provide classical pharmacokinetic treatments of specific drugs will not be included here. Dedrick et al.[71] used in vitro metabolic data and a pharmacokinetic model based on "flow limitation" to successfully predict plasma concentrations of Ara-C and Ara-U in man following intravenous administration of the parent compound. Tsuchiya and Levy[72] pointed out that when drugs are eliminated by parallel first order and Michaelis-Menten kinetics, such as in the case of the salicylates, the ratio of plateau level to dose increases with increasing dose. This might be the cause of adverse and toxic effects of these drugs during chronic therapy. DiSanto and Wagner[73] showed that plasma and tissue level data for methylene blue in a dog appeared to be better fitted with a nonlinear heterogeneous, one-compartment open model than with a classical linear two-compartment open model. Through simulated data, the same authors[74] cautioned that due to limitation of assay sensitivity, data which were derived from a non-linear tissue binding model might, however, be erroneously assigned to the classical linear two-compartment open model. A consective first order absorption (dissolution and/or stomach emptying followed by transfer of drug across the gastro-intestinal membrane) two-compartment open model has been proposed[75] for propoxyphene. One of the two first order absorption constants could well be ascribed to the established first pass effect of the drug.[76]

Distribution - Various studies on the distribution of drug from plasma to the target tissue area have been reported. The stratum corneum was shown

to be a readily accessable compartment for griseofulvin, which accumulates rapidly in the skin after oral administration, and falls off more rapidly in skin than in blood upon discontinuation. The distribution of griseofulvin into skin is somewhat dependent on the climatic environment[77]. Isosorbide administered orally to rabbits also rapidly enters the eye where the drug is more rapidly eliminated than in plasma[78]. Distribution of ampicillin and cloxacillin into synovial fluid was rapid following oral administration of the antibiotics. The total level of cloxacillin (highly protein bound) in synovial fluid was much lower than that in serum, although the levels of free drug were similar in both fluids. Total levels of ampicillin in the two fluids were similar because the drug is not highly protein bound[79]. Graham and Rowland[80] found that salicylic acid concentrations in saliva were proportional to those in plasma after oral administration of aspirin, and that salicylic acid had similar half-lives in both fluids. This study suggested that measurement of salivary concentrations of salicylic acid may be useful in biopharmaceutical studies of aspirin and other salicylates.

Metabolism - There were two very important contributions toward the understanding of metabolism in relation to pharmacokinetics in the past year. Wan et al[81] showed that the kidney makes an appreciable contribution to the total body metabolism of benzoic acid, salicylic acid and p-aminobenzoic acid in animals. The renal contribution toward overall metabolism was quantitated by the utilization of apparent clearances and true renal clearances of the metabolites. Levy and coworkers[82] showed that hydroxylated metabolites of diphenylhydantoin and phenylbutazone inhibit the elimination of their parent drugs in the rat. This product inhibition mechanism may be operative in the dose-dependent elimination kinetics of drugs where the rate of decrease of drug concentrations at a given plasma concentration in the post-distributive phase decreases with increasing dose. Various drug interactions in which metabolism is affected have also been described. Pretreatment with MK-486, a peripheral decarboxylase inhibitor, decreases the elimination of L-dopa[83], thereby increases patient peak plasma dopa levels three-fold, while simultaneously decreasing overall excretion and urinary levels of dopamine and homovanillic acid. Oral contraceptive use was found to decrease serum folic acid and vitamin B_{12} levels[84], prolong the plasma half-life of antipyrine, but not that of phenylbutazone [85]. A transient enhancement of warfarin-induced hypoprothrombinemia by triclofos was observed, which can be accounted for by a displacement of warfarin from human albumin by trichloroacetic acid, a metabolite of triclofos[86]. Champion et al[87] showed that aspirin does not alter indomethacin pharmacokinetics in man, thus providing negative evidence for a previously reported drug interaction between the two drugs.

Effects of Physiologic and Pathological Factors on Elimination - Water loading was shown to increase the fraction of drug excreted free and the apparent renal clearance of sulfamethazine, sulfisomidine and sulfathiazole. The metabolic clearance of these drugs, however, was unaffected[88]. Lower plasma phenacetin concentrations were found in cigarette smokers than in controls[89], possibly due to increased metabolism of the drug in the gastrointestinal tract or during first pass through the liver. The

metabolism of salicylamide in volunteers during episodes of pyrogen-induced fever shows a significant reduction in the half-life of excretion of metabolites and alteration in the proportion of metabolites excreted[90]. Elimination of chloramphenicol in patients with liver cirrhosis correlated well with serum albumin and serum bilirubin concentrations but not with prothrombin activity[91] (all three parameters are biochemical tests indicative of extent of hepatic cirrhosis). Elimination of thiamphenicol, however, does not correlate with any of these tests. In renal failure, compensatory elimination of propranolol metabolites is achieved by a higher faecal excretion[92]. Increase in serum half-life of chlorpropamide is not proportional to the reduction of glomerular filtration rate but is related to the urinary clearance of the drug, which takes place mainly at the renal tubules[93].

Biliary Excretion - Studies on the biliary excretion of lidocaine[94], spironolactone[95] and riboflavin[96] were reported. Dunn and Beck[97] showed that while the bile concentration and the blood level of Telepaque® are related hyperbolically, the total amount of drug excreted per unit time is linearly related to bile flow. Intravenously administered diazepam elicited clinical effects which subsided after two hours to be followed by recurrence at about six hours. This recurrence is matched by an increase in plasma level of the drug, probably due to enterohepatic cycling, and is supplimented by a slow build-up of an active metabolite[98]. The effects of drug metabolism inducers and inhibitors on the biliary excretion of 3-methylcholanthrene and its metabolites were studied, and it was concluded that metabolism is the rate-limiting step in the biliary excretion of the parent drug[99]. Hirom et al.[100] showed that there is a species dependent threshold molecular weight for appreciable biliary excretion of anions. Similarly, biliary excretion of sulfonamides[101] requires a threshold molecular weight, although the extent of excretion above this threshold is not directly proportional to molecular weight.

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