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SECTION EDITORS
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PREFACE

This fourth volume of the series is, again, the product of combined efforts of many people. Editors add, retain, discontinue or modify subjects to be covered, choose authors, and labor to meet deadlines. Authors select, evaluate and interpret many articles and condense this mass of material into a few pages. All this is directed toward achieving the aims previously stated: brief, critical summaries of significant contributions to Medicinal Chemistry published during the past year and projections of future developments. Many changes in contributors, topics and viewpoints take place each year; this, we feel, is as it should be.

This volume represents considerable effort on the part of busy, productive scientists. I take this opportunity to thank each of them sincerely and hope that other members of the Division will find occasion to do the same.

Fort Washington, Pennsylvania
June, 1969

Cornelius K. Cain

Section I - CNS Agents

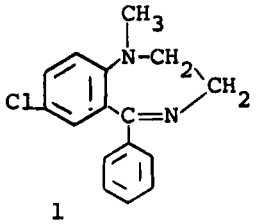
Editor: Scott J. Childress, Wyeth Laboratories, Inc. Radnor, Pennsylvania

Chapter I. Antipsychotic and Anti-anxiety Agents

Irwin J. Pachter and Alan A. Rubin
Endo Laboratories Inc., Garden City, New York

Newer Compounds in Clinical Trial

The initial promise noted in the 1967 Annual Reports for medazepam (RO 5-4556, 1) has been substantiated by new publications during 1968. The pharmacology employed in selection of the compound[1,2] as well as associated toxicological[1] and metabolic studies[3] have been published. Medazepam alleviates anxiety and tension in children[4] and adults[5-10] with minimal sedative side effect and without impairment of working capacity or normal alertness.



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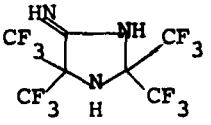

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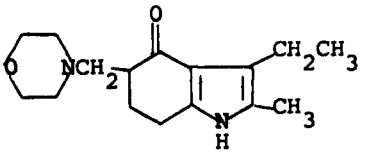

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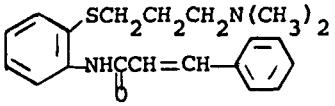
EXP 338 (2), a novel structural type, was an orally effective centrally-acting skeletal muscle relaxant in experimental animals. In patients suffering from acute anxiety and depression, results with 10.5 mg. of EXP 338 compared favorably with those obtained with 75 mg. of chlordiazepoxide.[11] In another study involving spastic multiple sclerosis patients its muscle relaxant effects were variously accompanied by increased alertness, euphoria, insomnia, irritability, increased libido and paranoid psychotic reaction.[12]

Molindone (3) is an effective antipsychotic drug.[13,14] In a double blind comparison study it proved to be of similar milligram potency to trifluoperazine.[15] Possible antidepressant properties attributed to the drug by others[13,16] were ascribed to its activating and stimulating effects.[15]

It has been suggested that schizophrenia may be an immunologic disorder.[17,18] In addition, some correlation has been reported to exist between central serotonin antagonist activity of the phenothiazines and their tranquilizing action. Cinanserin (4) which displays both antiserotonin and immunosuppressive properties was tested in chronic schizophrenics. It had no beneficial effect.[19]



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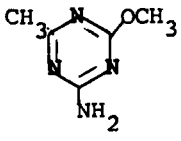

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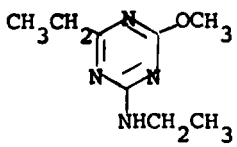

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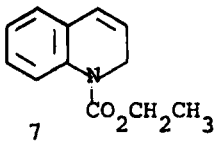
Clinical effectiveness of 5 in the treatment of chronic schizophrenics[20] led to the synthesis and pharmacological evaluation of a more potent analog EEM (6). EEM appears to act through centrally mediated cholinergic mechanisms.[21]

1-Carbethoxy-1,2-dihydroquinolines and 1-carbethoxy-1,4-dihydroquinolines

were found to be potent central nervous system depressants and irreversible inhibitors of the adrenergic α-receptor.[22] One compound, designated as BC-347 (7), inhibited conditioned avoidance in rats and showed tranquilizing properties in mice, rats, cats, dogs and monkeys. Unlike other antipsychotic agents, it did not have antiemetic activity in dogs. In chronic schizophrenics it showed a low degree of antipsychotic effect. Side effects were troublesome and BC-347 was not considered promising for treatment of schizophrenia.[23]



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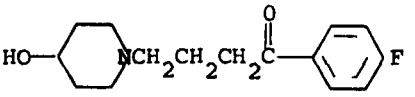

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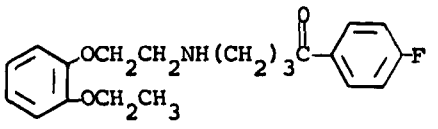
The butyrophenone AL-449 (8) also lacked antiemetic action in animals in an otherwise typical major neuroleptic profile. In chronic schizophrenics it showed practically no evidence of antipsychotic action.[24,25]

Further work on the butyrophenone CI-601 (9) confirmed earlier observations that the drug has definite antipsychotic properties, but that side effects were more troublesome than with existing therapeutic agents.[26,27]



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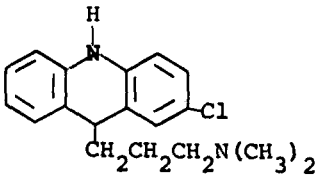

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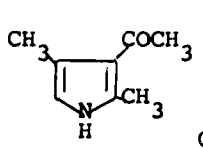
Clomacran (SKF 14336, 10) in further studies was found to be as effective as trifluoperazine with somewhat fewer side effects[28] and almost identical to chlorpromazine but with a relatively higher incidence of extrapyramidal symptoms.[29]

Of many substituted pyrroles showing CNS depressant effects in rodents, 3-acetyl-2,4-dimethylpyrrole (11) was selected for study as a tranquilizer and muscle relaxant in man. Side effects precluded going to doses large enough to observe desired effects.[30]



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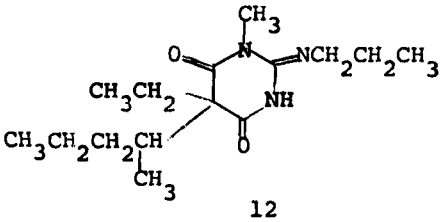

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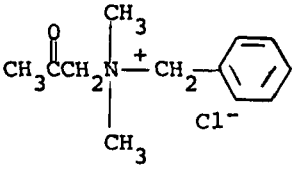


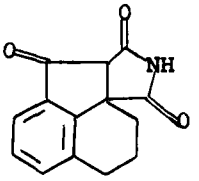
13

Preliminary studies with W-1867 (12) in hospitalized mental patients indicated little therapeutic efficacy.[31]

BL-KR 140 (13) had no antipsychotic or neuroleptic effect in chronic schizophrenics.[32] The possibility that 13 might possess antidepressant effects[32] led to its trial in chronic anergic schizophrenics stabilized on

neuroleptic agents. At doses as high as 1200 mg. per day, improvement and symptom change with 13 was negligible.[33]

The compound CM-6 (14) which showed anticonvulsant activity in animals,[34] was reported to have psychotropic effects of a tranquilizing type in schizophrenic patients. In more extensive evaluation,[35] the drug markedly activated schizophrenic symptomatology. Hallucinatory activity and delusions intensified, insight was lost and hostile belligerence increased.



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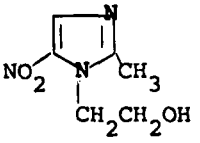

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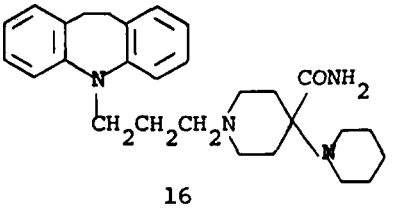


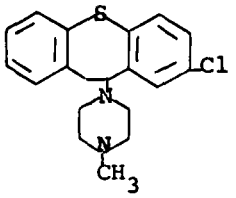
16

Such symptom provocation may be useful in treatment of chronic therapy-resistant schizophrenics who are apathetic, anergic and depressed. After activation, treatment with psychotropic drugs is sometimes more effective.[36] Metronidazole (15), used widely in the treatment of trichomoniasis and more recently in the treatment of alcoholism,[37] was also found to increase agitation, delusions and hallucinations when administered in large doses to schizophrenics in need of activation.[36]

Carpipramine (16), combining typical structural features of the antidepressants and the butyrophenones, is less potent as a reserpine antagonist than imipramine.[38] It exhibited no antidepressant effect in man but was effective in chronic schizophrenics where communication with environment was a particular problem.[38]

A previous report[39] that a steroid, 6β-hydroxy-3,5-cyclopregnan-2-one (neurosterone), is of value in treatment of psychiatric patients was not substantiated in a new controlled study. Neurosterone was not superior to placebo and was clearly inferior to chlorpromazine.[40]

Octoclothepine (17) showed antipsychotic action in schizophrenics. Side effects included akathisia, hypotension and tachycardia.[41] On the basis of electroencephalographic studies in cats[42] and rabbits[43] it was predicted that the related compounds dibenzoxazepine (18) and clothepine (19) will also prove to be major neuroleptics.



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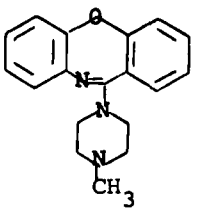

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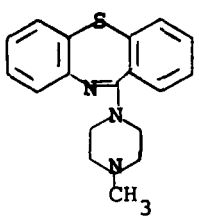
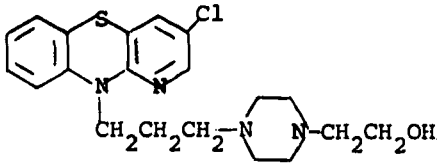


19

The synthesis of cloxypendyl (20) and many of its congeners was described. It is an effective compound in the treatment of schizophrenia.[44]

Doxepin, (21) previously studied as an antidepressant, proved effective as a tranquilizer in a double blind comparison with chlordiazepoxide.[45]



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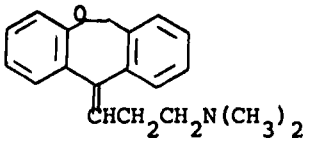


21

Thiothixene, useful in treatment of schizophrenia, was also found useful in treating neurotic patients.[46] In another study, it was found as effective as the perphenazine-amitriptyline combination in treating psychoneurotic depressive symptoms.[47]

Tybamate, tested as an antipsychotic agent, was not effective.[48]

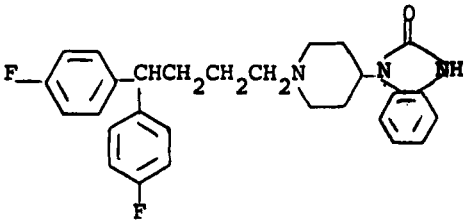
Newer Structures of Interest

Pimozide (22) resembles haloperidol in its profile in experimental animals but is longer acting and relatively less toxic.[49]

Peradithiepine (23) is a potent depressant in mice with a therapeutic ratio 15 times as favorable as that of chlorpromazine.[50]

Many new phenylpiperazine derivatives with combined neurological and hypotensive properties have been described.[51-54] Of these, AF 1161 (24), a CNS depressant with weaker adrenolytic action than chlorpromazine but stronger antiserotonin activity has been recommended for clinical trial as a psychotropic drug.[54]

Benzoctamine (25) has been designated as a psychopharmacologic agent with sedative and muscle relaxant properties.[55]



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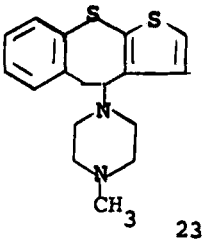

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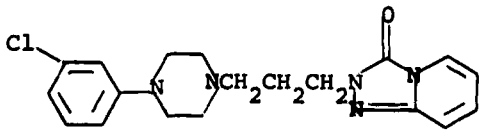

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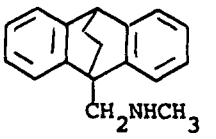


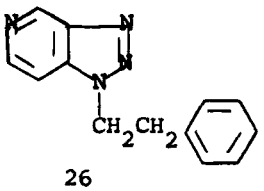
25

A compound designated as PCA 9 (26) blocks conditioned avoidance in rats and produces tranquilizing and sedative effects in animals at doses which do not affect motor coordination.[55]

Several 3-hydroxyindole derivatives have been found to reduce work rates in trained rats. These compounds are considered possible psychotropic

metabolites. 1-Methylindican (27) at 6 mg./kg. intraperitoneally caused a work rate reduction of over 85%.[56]

Compound 28 reduced locomotor activity at intraperitoneal doses of 1-2 mg./kg. Related substances also showed significant activity.[57,58]



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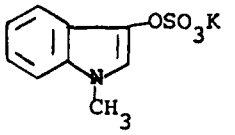

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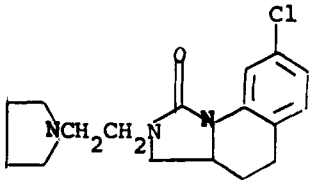

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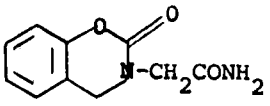

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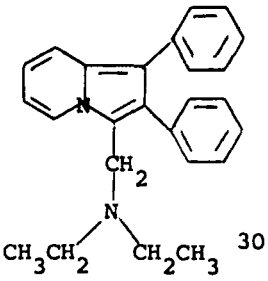

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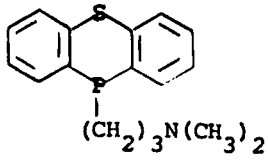

31

Compound 29 decreased spontaneous activity in mice while antagonizing reserpine-induced ptosis.[59]

Rats lost aggressiveness and mice exhibited pronounced depressant effects when 10 mg./kg. of compound 30 was given intraperitoneally.[60]

Phosphorus-containing relatives of the phenothiazine tranquilizers such as 31 show relatively weak depressant effects.[61]

Phenothiazine tranquilizers, when quaternized with phenacyl bromide oxime, are reported to give products almost as potent as the parent drug but which produce less catatonia and other activity associated with extrapyramidal side effects in man.[62] Quaternization of chlorpromazine with methyl chloride, on the other hand, produces a compound with markedly more toxic and less sedative effects in rodents.[63]

Benzodiazepines and related compounds have been the subject of several important papers which appeared during 1968. Structure-activity relationships were reviewed.[64] The syntheses of 55 3-substituted-1,4-diazepines and their activities relative to oxazepam were described.[65] Trifluoromethoxy-and trifluoromethylthio-1,4-benzodiazepines were prepared.[66] 1,4-Benzodiazepines with double bonds in the 3,4-position were found to be markedly less active than those unsaturated in the usual 4,5-position.[67] Chemistry and pharmacology of an extensive series of tetrahydroisoquinobenzodiazepines (isoquinazepon series) were reported;[68] certain indications of possible CNS stimulant activity are exhibited by members of this series in addition to the usual profile of the 1,4-benzodiazepines.

Metabolic Studies on Antipsychotic and Anti-Anxiety Agents.

Trifluperidol decreases serum cholesterol through interference with conversion of 7-dehydrocholesterol into cholesterol rather than through desmosterol accumulation.[69]

The metabolism of thiothixene with emphasis on tissue distribution and excretion was reported.[70] One major metabolite appears to be the piperazine-N-demethylated analog.

In common with findings with other phenothiazines, sulfoxide formation occurs during trifluoperazine metabolism.[71]

Metabolism of phenothiazines and related compounds was the subject of an entire issue of Agressologie.[72]

Liver supernatants from dogs,[73] rats and mice[74] convert diazepam into N-desmethyldiazepam, N-methyloxazepam and oxazepam. Urinary metabolites of chlordiazepoxide in the rat include four compounds which have a hydroxyl function on the C-5 phenyl ring.[75]

A lack of correlation of serum levels with clinical effects previously observed for chlorpromazine and imipramine was also observed for meprobamate, tybamate and pentobarbital sodium.[76]

Central Synaptic Transmission

The reviews of Ungar and Irwin[77] and Aprison and Werman[78] are recommended as references for the consideration of central synaptic transmission. Both critically evaluate the recent literature and apply fundamental criteria for identification of transmitter candidates. In essence, these criteria include (1) presence at presynaptic nerve endings, (2) release following presynaptic nerve stimulation and (3) similarity between alleged transmitter and presynaptic nerve stimulation at postsynaptic sites.

The only substance reasonably satisfying these conditions is acetylcholine as it affects spinal cord Renshaw cells. At a recent symposium, however, the existence of Renshaw cells as discrete units was challenged on electrophysiological[79] and histochemical[80] grounds but also staunchly defended.[81] Little evidence has been presented to support a widespread central transmitter function for acetylcholine. Krnjević[82] suggested that the slow, prolonged cortical iontophoretic effects of acetylcholine are consistent with a general facilitatory rather than a direct transmitter action.

The central transmitter status of other cerebral amines is less imposing than that of acetylcholine. Evidence implicating catecholamines and serotonin is largely circumstantial falling short of criteria 2) and 3) because of methodological deficiencies. Two reviews have considered this subject, one[83] describing the relevant profiles of norepinephrine, dopamine and serotonin and the other[84] indicating various research strategies relating biochemical behavioral events. The popular concept that norepinephrine is normally alerting[85] and possibly elating in certain affective disorders[86] has been challenged by a number of investigators. Dewhurst[87,88] has proposed that cerebral amines are characterizable as excitant (type A, represented by tryptamine), depressant (type C, represented by catecholamines) or sequentially excitant then depressant (type B, represented by serotonin). Methysergide, in this context a specific competitive antagonist of type A receptors, has benefited a few patients with mania.[89,90] Many obvious questions arise in connection with this theory, particularly in regard to explanations for the pharmacological actions of such agents as phenothiazines, α-methyltyrosine and p-chlorophenylalanine. Nevertheless, Dewhurst's theory has thus far been acclaimed,[91] criticized[92] and vigorously defended.[93]

Mandell and his colleagues[94,95] have also speculated that norepinephrine has a depressant function. Their data indicate that imipramine-induced

hyperactivity in chicks is associated with decreased rather than increased availability of brain norepinephrine at postsynaptic receptor sites.

There has been increasing recognition accorded the independent activity of dopamine over the past year. Creveling et al[96] have suggested that dopamine, and not norepinephrine, is responsible for reawakening reserpinized mice and Pscheidt and Himwich[97] have shown that imipramine can block dopamine uptake at central neurons. Brain dopamine levels were found to correlate positively with EEG arousal responses in rabbits treated with tetrabenazine and dihydroxyphenylanine.[98] Nyback and Sedvall[99] suggest that chlorpromazine accelerates the synthesis of dopamine and not norepinephrine whereas Barbeau[100] reaffirmed the finding that chlorpromazine enhances the metabolism of dopamine to homovanillic acid.

Other recent reports which indirectly tend to weaken the concept that norepinephrine is the sole alerting neurohumor indicate that (1) imipramine hyperactivity may result from blocking uptake and reducing nervous impulse flow in central serotonin neurons;[101] (2) drugs which inhibit uptake of catecholamines also block serotonin accumulation in rabbit brain stem preparations;[102] (3) the increase in overt stimulation caused by 5-hydroxytryptophan may be associated with impaired norepinephrine synthesis rather than increased norepinephrine release[103] and (4) norepinephrine and dopamine inhibit electrical activity of central neurons as determined microelectrophoretically[104] although another study indicated that norepinephrine does cause neuronal excitation.[105]

Although considerable evidence has accumulated recently in support of a central transmitter function for serotonin in molluscs,[106-108] collection of appropriate data in regard to mammalian synapses has been more elusive. There is relatively little information available concerning release and recovery of serotonin from specific central sites or similarity of action between serotonin and its alleged transmitter counterpart. Some progress has been made in stimulating central neurons by microelectrophoretic techniques.[109,110] But other difficult problems exist especially as they relate to cerebral tryptamine receptors[111] and inhibition of serotonin biosynthesis.[112,113]

Nevertheless, the role of serotonin in influencing behavior was impressively advanced by Jouvet's investigations[114,115] on the process of sleep. Destruction of serotonin neurons in the raphe system or inhibition of serotonin biosynthesis result in insomnia. Paradoxical (REM) sleep, the functional state following slow wave (NREM) sleep, may depend upon a more complex amine system involving serotonin, norepinephrine and acetylcholine. Recent reports[116,117] have corroborated the relationship between serotonin and slow wave sleep.

Other transmitter candidates considered in the past year include the inhibitors γ-aminobutyric acid[118] and glycine.[78,119] The latter is present in high concentrations in the spinal cord in association with synaptic functions and interneurons[119] and has been shown to inhibit central neurons.[120] Four prostaglandins were found to be widely distributed throughout the dog brain but evidence supporting a transmitter function is not presently available.[121] Finally, an actomyosin-like protein with magnesium or calcium activated adenosine triphosphatase activity has been isolated from whole rat and cat brains.[122] This substance may mediate nerve impulse conduction.

In their provocative review, Ungar and Irwin[77] suggest that the

mechanisms characterizing complex interactions in brain neurons be reassessed. They contend that synaptic function may be modified by substances (modulators) which either affect synthesis, storage or release of transmitters at presynaptic endings or excitability at postsynaptic structures. Toman and Sabelli[123] believe that the term modulator should be extended to include all endogenous brain substances capable of altering neuronal function. In this context, a modulator-receptor system could provide a more generalized "central linkage" embracing synaptic transmission as well as mimesis, facilitation, release, etc.

Psychotic Disorders

Pauling[124] recently introduced the concept of orthomolecular psychiatry in an attempt to redefine the basis of mental disease and the treatment thereof. He suggests that aberrant mental function and behavior may be due to inadequate brain levels of essential nutrients (e.g., vitamins, amino acids, fatty acids, etc.). Orthomolecular psychiatric therapy, therefore, would provide optimum concentrations of these vital substances in order to correct alleged cerebral deficiencies. Diagnostic methods for determining optimum levels of a pertinent nutrient for individual patients are not yet available. One critic[125] has already taken issue with these views. Other investigators[126] have independently considered the possibility that abnormal plasma levels of free fatty acids may influence the erratic dietary intake of schizophrenic children.

Kety[127] has reviewed the evidence relating to the catecholamine transmethylation and adrenochrome hypotheses of schizophrenia advanced by Osmond and Smythies.[128] The former concept is still intriguing although in need of more supportive data while the latter is all but dismissable as a viable thesis. An analogous concept associating indoleamine transmethylation with schizophrenic behavior has received support from Spaide et al,[129] although Faurbye and Pind[130] reported that at least one methylated indole, bufotenin, is not specific for schizophrenic urine. Favorable consideration to the mechanisms involving metabolic aberrations in the synthesis of catecholamines and serotonin has been presented by Shulgin et al[131] and Faurbye.[132]

Reynolds[133] revived the hypothesis that epilepsy and schizophrenia are biologically antagonistic disorders. He cites reports indicating that anticonvulsant drugs may precipitate a schizophrenia-like pyschosis whereas phenothiazines may cause epileptic fits. As cited above, Angrist et al[35] found that CM-6 (14) an experimental drug with prominent anticonvulsant properties in animals activated psychotic symptoms in schizophrenic and other psychiatric patients. And Guerrero-Figueroa et al[42] have shown that antipsychotic drugs facilitate electrical activity in the cortex and limbic system of normal and epileptic cats often causing frank convulsions.

The investigations of Heath and his colleagues[18] continue to provide data indicating that schizophrenia is an immunologic disorder. Passive transfer of the γ-globulin taraxein from schizophrenic patients to normal volunteer recipients or rhesus monkeys resulted in acute psychotic episodes. Similar experiments with γ-globulin fractions from psychotic patients other than schizophrenics produced negative results suggesting that taraxein is specifically associated with schizophrenia. Lehmann[134] reviewed Heath's hypothesis with perception and compassion enumerating clearly the attractive as well as the unresolved features.

Other investigators have attempted to relate an abnormal plasma factor

with psychotic disorders. In a recent cooperative study[135] two laboratories, using their own isolation and assay procedures, consistently identified a protein factor in schizophrenic serum. The physical characteristics of this factor apparently differ appreciably from those described for Heath's taraxein. Other plasma correlates of psychosis mentioned in the literature of the past year include elevated plasminogen[136] and thyrotrophic hormone[137] levels, abnormal lymphocytes[138,139] and antigammaglobulin activity.[140] Orlovskaja et al[141] found that injection of normal and schizophrenic serum into laboratory animals resulted in different plasma profiles of corticosteroids, sugar and lymphocytes. A general review of Soviet activity in this area[142] indicated studies in progress concerned with lymphocyte morphology and erythrocyte permeability.

The search for relevant chemical correlates is still in an exploratory phase operating across a broad experimental front. It appears now that such a multifaceted approach will continue in the foreseeable future at least until a major discovery encourages a convergence of research energies. Despite all of the inherent methodological difficulties,[143] progress continues to be made in pursuit of an extremely elusive goal.

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Chapter 2. Antidepressants, Stimulants, Hallucinogens

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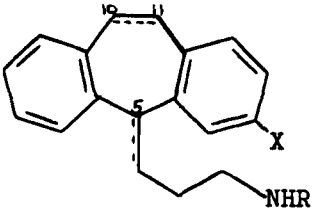
I. ANTIDEPRESSANTS

Chemistry; New Structures - A structure-activity study of dibenzocycloheptene derivatives showed protriptyline (1; $\Delta^{10,11}$, X=H, R=CH_3) to be the most potent compound in antagonising the effects of tetrabenazine (TBZ). Nortriptyline (1; Δ^5, X=H, R=CH_3) and nuclear-substituted compounds were less active[1]. The oximinoether 2 (CD 37B) possessed pharmacologic properties similar to those of the tricyclic thymoleptics and was claimed to have antidepressant action in man[2]. A pyridyl analogue (3a) of imipramine was more toxic and less potent as an antagonist of TBZ than the parent drug[3]. Carpipramine (3b), containing structural elements of imipramine and the analgesic drug pirinitramide, was compared to the former. Pharmacologically it possessed some of the properties of imipramine (including an analgesic action: cf.[4]) while clinically it was reported to alleviate the depressive state in schizophrenia[5]. A study of the charge-transfer spectra of various phenothiazine neuroleptics and tricyclic thymoleptics suggested that the differences in physiological action are not dependent on differing electron-donating properties[6]. Octahydroacridine derivatives (4) were reported to have imipramine-like pharmacological properties although the degree of activity was less than that of the standard drug[7]. A number of cyclohepta[b]quinolines(5) were assayed for antidepressant activity using the DOPA-potentiation test; compounds Y=Cl, $OCH_2CHMeCH_2NMe_2$ as well as Y=3-NO_2, R=$O(CH_2)_3NMe_2$ were among the more active[8].

Selective blockade of muricidal behaviour in the "killer" rat is claimed to be predictive of clinical antidepressant activity[9]. Using this in combination with the rotarod test, it was found that in a series of benzothiazepinones 6a and 6b were slightly more active than the related drug thiazesim (6c, Altinil^R)[10]. Anti-reserpine activity was found in a number of benzoxazinones, compound 7 being the most potent member of the series[11]. Based on its novel and complex pharmacological profile, compound 8 (AF-1161) was suggested as a possible antidepressant[12]. A detailed study of 3-β-phenoxytropanes indicated that 9 (X=H) in particular possessed some of the properties of amphetamine (hypermotility) and the tricyclic antidepressants (reserpine-and TBZ-antagonism, potentiation of the pressor action of norepinephrine)as well as a marked central but weak peripheral anticholinergic effect[13]. The pharmacological properties of N-3,4,5-trimethoxybenzoyl heptamethylenimine were claimed to closely resemble those of the tricyclic drugs[14].

There were several reports of new MAO inhibitors. The following compounds had, in addition to this action, some of the pharmacological properties of the antidepressants. Compound 10, an analogue of pargyline, exhibited anti-reserpine and excitatory effects in mice[15]. In a series of cyclic derivatives of N-aminoephedrine, 11 had anti-reserpine effects and

was relatively non-toxic[16]. A series of 2-phenylcycloalkylamines (12), was studied[17]. Only tranylcypromine (12, n=0) inhibited MAO and caused hypermotility, although the higher homologues (n=2,3) also caused reserpine-reversal. Compounds of type 13 were highly active in the DOPA-potentiation test in mice but the activity could not be correlated with their in vitro MAO inhibitory effect[18]. A related compound (14, M & B 9302) is an extremely active MAO inhibitor and has anti-reserpine properties in a number of species[19]. The propargylamine 15 (E-250), claimed to be a clinically effective antidepressant, was reported to inhibit the effects of tyramine, a property which could obviate the problem of the "cheese syndrome"[20].



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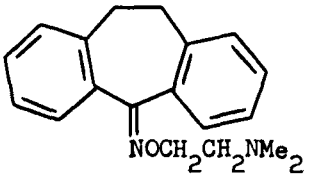

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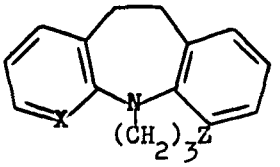
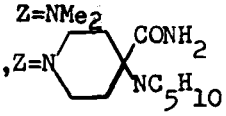
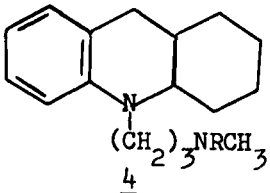


3a X=N, Z=NMe2



3b X=CH, Z=N



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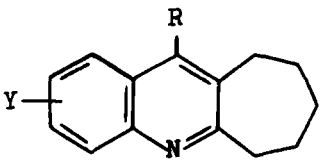

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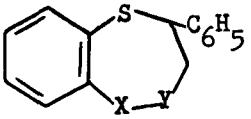
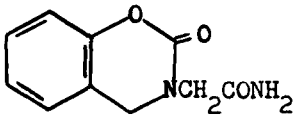


6a X=C=O, Y=NC2H4NMe2
6b X=C=O, Y=NC2H4NEt2
6c X=NC2H4NMe2, Y=C=O



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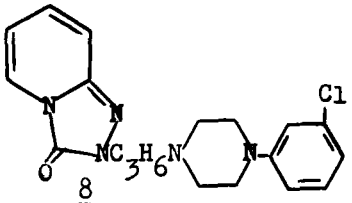
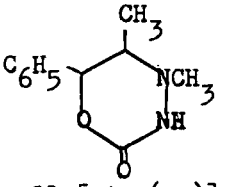


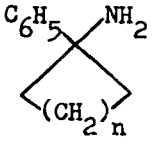
8

9 X=H, m-CF3, p-Cl

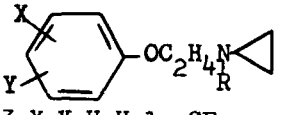
10



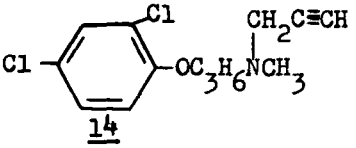
11 [cis-(−)]



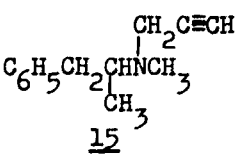
12 (n=0−3)



13 X,Y=H,Halo,CF3
R=H, CH3



14



15

Biology - A monograph on behavioural pharmacology[21] and an extensive review of the thymoleptic agents[22] have appeared, the latter covering methods of pharmacological evaluation, biochemical mode of action and metabolic fate of imipramine-like drugs. A comprehensive survey of the comparative pharmacology of imipramine and chlorpromazine was published, with the conclusion that both drugs have a similar spectrum of activity, being mild analeptics at low doses and depressants at high doses[23]. A similar observation was made from a comparison of the pharmacological profiles of the tricyclic thymoleptics to the neuroleptics[24].

Newer test procedures included the potentiation of yohimbine-induced behavioural patterns in dogs[25] and antagonism of reserpine in the Pencil Fish[26]. The former test offered promise as a good indicator of clinical efficacy. The specificity of the reversal of reserpine hypothermia for the evaluation of antidepressant activity was affirmed[27]. McKearney, however, reported that the tricyclic thymoleptics do not antagonise the behavioural effects of reserpine or TBZ. Over a wide range of doses imipramine and desipramine failed to antagonise the effects of these amine depletors on schedule-controlled performance in the rat[28].

Based on their ability to inhibit catecholamine uptake, new applications have been suggested for the thymoleptics. Imipramine, amitriptyline and their N-desmethyl analogues markedly inhibited experimentally-induced inflammation in rat paws. The effect was reduced by adrenalectomy or by pretreatment with reserpine or inhibitors of norepinephrine (NE) biosynthesis[29]. In animals and man the administration of protriptyline with NE decreased intraocular pressure, indicating a possible use in glaucoma[30].

A vast amount of material has appeared concerning the role of the biogenic amines in behavioural states. Two general reviews were published[31,32] as well as a survey of recent work on adrenergic mechanisms in the central action of imipramine-and chlorpromazine-like drugs[33]. Kety's perceptive comments on the field[34] merit attention. Some basic objections to the generally held theory of cerebral amine function, as set forth by Brodie, were raised and an alternative one was proposed by Dewhurst[35]. In brief, the new theory reverses the respective roles of norepinephrine and serotonin (5-HT) as excitant and depressant substances and holds that it is the lipid solubility which determines the type of action of a given amine; alteration of the side-chain affects potency and duration of action. Antidepressants could thus owe their behavioural effects to an alteration of indoleamine rather than catecholamine metabolism. Work reported by other groups gave some support to the theory. 5-Hydroxytryptophan was active in the anti-muricidal test for antidepressant activity[9] while tryptophan itself was reported to be as effective as electroshock in patients with severe depression[36]. Imipramine was found to retard amine depletion from central 5-HT neurons[37], although desipramine and protriptyline blocked the uptake of NE into the central NE neurons[38]. p-Chloro-N-methylamphetamine (CMA), reported to have clinical antidepressant rather than amphetamine-type stimulant activity, selectively altered indoleamine rather than catecholamine metabolism in animals and released 5-HT from its depots in man[39]. It was suggested that imipramine may block the access of behaviourally-depressing NE to post-synaptic sites. A similar mechanism has been proposed for the phenothiazines and this could explain their

efficacy in certain types of clinical depression[40]. Phenethylamine, rather than the catecholamines, may be the neurohumoral ergotropic agent in mammalian brain[41].

Imipramine blocked the uptake of dopamine at central aminergic neurons in the rat[42]. Lithium inhibited the electrically-induced release of NE and 5-HT in brain slices[43] and did not interfere with the transfer rate of Na^+ from blood to brain tissue[44]. Data were reported which suggested that the therapeutic effect of electroshock treatment may be due to increased levels of brain amines[45] or to an increase in NE turnover rate[46]. In a study of catecholamine turnover rates in mouse brain it was found that neuroleptics have a predominant influence on dopamine metabolism while antidepressants selectively affect NE metabolism[47]. A higher rate of NE synthesis was found in the forebrain of "mouse-killing" rats over that of controls[48]. Imipramine blocked the muricidal behaviour[9] and also lowered NE turnover[48].

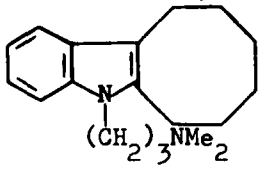
Pharmacological and clinical effects of MAO inhibitors were discussed[49]. The clinical antidepressant activity of these drugs was claimed to be related to the degree of inhibition of NE re-uptake, the same mechanism by which the tricyclic antidepressants are considered to exert their effects; only limited data were given[50]. Based on a study of NSD 2023 (γ-morpholinobutyrophenone), it was suggested that the anti-reserpine action of MAO inhibitors is unrelated to the enzyme inhibition[51]. Combined administration of reserpine and MAO inhibitors to rabbits produced EEG, behavioural and biochemical changes which could explain the therapeutic effects of this regimen in the management of endogenous depression[52]. Agents such as the MAO inhibitors which alter the availability or production of hypothalamic amines, such that 5-HT is proportionally higher than catecholamines, cause blockade of ovulation[53].

A detailed study of the absorption and distribution of imipramine and the formation of 14 metabolites was reported[54]. In contrast to an earlier report, data were presented which confirmed that desipramine is the pharmacologically active metabolite of imipramine[55,56]. Urinary excretion of 3-methoxy-4-hydroxyphenylglycol (MHPG), the major metabolite of NE in brain[57], was significantly lowered in depressed patients[58].

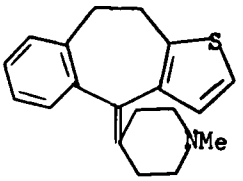
Clinical - Several reviews and articles on the nature and therapy of depressive disorders were published in 1968 [59-63] as well as a useful introductory text on clinical psychiatry[64]. Irwin[65] has proposed a rational framework for the clinical evaluation and use of psychoactive drugs. He noted that patients with depression exhibit a wide variety of behaviour and that the different forms of the disease require different classes of drugs. Mendels[66] likewise remarked that "few terms in psychiatry are used with as many different meanings as depression". The importance of using a suitably large sample size in clinical studies of drug evaluation was stressed[67]. The foregoing are a caveat for the interpretation of the clinical reports discussed below.

No new antidepressants were introduced commercially in the U.S. in 1968; iprindole (16) was marketed in Britain. A review of the recent literature of this drug showed it to be effective and relatively free from

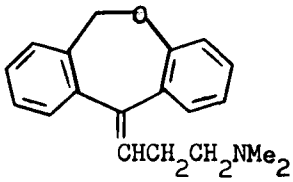
side-effects[68]. Pharmacological studies indicated that its mechanism of action is different from that of imipramine[69]. Psychoactive properties were seen with compound 17 when tested in schizophrenics with depression[70]. Another tricyclic agent, doxepin (18), was as effective as amitriptyline in a double-blind trial[71]. Thiazesim (6c) was effective in a number of depressive states and produced only mild side-effects[72]. The urazole derivative (19) showed promising antidepressant properties[73] and was claimed to be as effective as imipramine[74]. Compound 20 exhibited a profile of activity in animals quite similar to that of imipramine, being in some respects more potent. It was, however, of no specific value in patients with endogenous depression, thus raising the question of the validity of the generally-used animal screening procedures[75]. Similar disappointing results were obtained with 21[76]. A novel structure (22) when tested as a muscle relaxant was found to produce increased alertness and euphoria and was suggested as a possible antidepressant[77]. The pharmacological properties of the benzimidazolone 23 resembled those of the thymoleptics and the compound also possessed little sedative and anticholinergic effects. Preliminary clinical trials demonstrated its efficacy[78]. Convulsive therapy with flurothyl (hexafluorodiethyl ether) compared favourably with electroshock in a controlled trial[79].



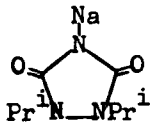
16 iprindole



17 BC-105



18 doxepin



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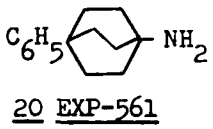

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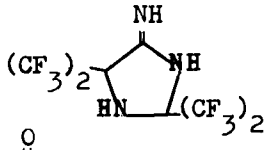
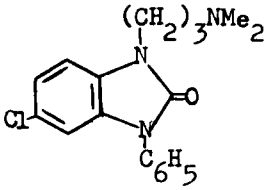


21 BL-KR140

22 EXP-338



23 AW-14,2446

Lithium carbonate has been extensively studied as a therapeutic agent; two collections of articles have appeared.[80,81] Its role in the treatment of mania is generally accepted but its value in depression[82] is less clear. Certain cases did respond favourably[83] while other workers reported that Li had little of the thymoleptic properties of imipramine in acute endogenous depression (ref. 80, p. 487). The drug does appear to be of definite value in preventing mood swings in recurrent affective disorders[80].

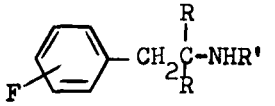
The renewed interest in the use of neuroleptics in depression has cast doubt on the view that the clinical effects of the tricyclic antidepressants and the phenothiazine antipsychotics are distinct[84]. Chlorpromazine was found to be equivalent to imipramine in cases of primary

depression[85] and, when given in high dosage, superior to placebo[86]. Beneficial effects were also obtained with thiothixene [87] and other agents generally classed as tranquilisers[88].

Knowledge of the role of the adrenocorticosteroids in disordered behaviour[62,89-91] has contributed to a practical therapeutic result. Combined administration of dexamethasone with an antidepressant (imipramine, amitriptyline or isocarboxazid) gave a rapid onset of favourable action in 17 cases of depression (ref. 68, p. 27). Other memorabilia from the clinical literature included a proposed relationship between drug effects and patients' body height[92] and the state of the weather[93].

II. CENTRAL STIMULANTS

Chemistry - A number of fluorinated α,α-dialkylphenethylamines (24, R=CH_3, R'=H,CH_3) had stimulant properties in mice; an analogue (3-F, R=C_2H_5, R'=H) had anti-reserpine action[94]. Several benzhydrylpiperidinols (25), related structurally to pipradol (26), were potent stimulants in mice. One of the most active (25, R=CH_3) was also effective clinically[95]. Both the (R)- and (S)-enantiomers of pipradol were prepared and all of the central stimulant activity was found to reside in the (R)- form. Since the more active enantiomers of pipradol and amphetamine are not configurationally related, the result suggests that the mode of action of these stimulants may differ[96]. Compound 27 (R=CH_3, Am=piperidino), the most interesting member of a series of basically-substituted xanthones, had stimulant and analeptic properties similar to those of pentylenetetrazole together with a favourable therapeutic index[97]. A structure-activity study of picrotoxin and related naturally occurring lactones indicated that the absolute requirements for stimulant action are (i) a lactone function connecting C-3 and C-5 of the picrotoxane (28) skeleton; (ii) a carbonyl system cis to the fused ring and (iii) a bridgehead hydroxyl group at C-6[98]. A novel tricyclic structure (29) was reported to possess stimulant properties in a number of animal species together with anorexic, anti-reserpine, anti-TBZ and anti-muricidal actions[99].



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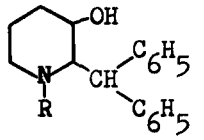

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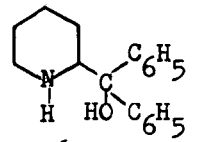

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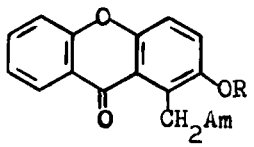

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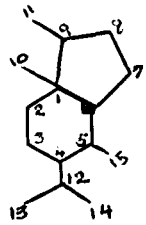

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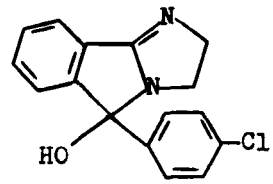
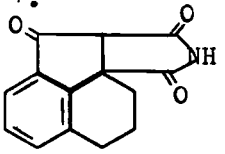


29 (42-548)

Biology - Theories concerning the mechanism of action of amphetamine in the CNS were discussed[100]. It is thought to act either directly on central catecholamine receptors or indirectly, through a release of NE or dopamine. Recent work supports the indirect process and it was held that the activity depends not on the actual level of brain NE but on its uninterrupted synthesis[101]. This view was supported by other workers who also found that the stimulant action of p-chloroamphetamine is mediated through 5-HT as well as NE[102]. It was suggested that the central actions of phenmetrazine, which are similar to those of amphetamine, are mediated through dopamine rather than NE[103]. Brain levels of amphetamine in rats were increased by tricyclic antidepressants and by phenothiazine tranquilisers; the former class prolonged and the latter inhibited the amphetamine-induced hyperthermia[104]. Desipramine was found to be about 4 times more potent than imipramine in enhancing the behavioural (non-discriminated avoidance) effects of a number of central stimulants[105].

The question of the possible facilitation of learning by central stimulants is complex and little new material can be added to last year's excellent review[106]. Recent work indicates that neither caffeine[107] nor magnesium pemoline [108] enhances learning rate.

Clinical - Aspects of the use and abuse of amphetamine were discussed [109,110]. Administration of the drug to volunteers in 120-220 mg/day caused a florid paranoid psychosis resembling paranoid schizophrenia[111]; the psychosis has also been compared to that seen in temporal lobe epilepsy[112]. Magnesium pemoline was reported to be effective as a stimulant in cases of fatigued subjects[113,114] but not in geriatric patients[115]. Pipradol caused a worsening of depression in affective states and its usefulness as a clinical mood elevator could not be supported[116]. A new anticonvulsant (30) possessed distinct psycho-activating effects[117].

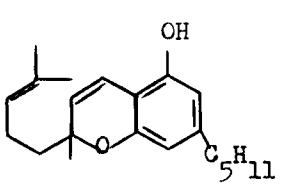


30 CM-6

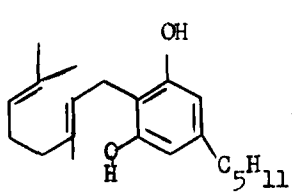
III. HALLUCINOGENS

Chemistry - Three interesting reviews on naturally occurring hallucinogens have been published[118]. Following the impressive advances made in the syntheses of the tetrahydrocannabinols (THC's), discussed previously in this series (Annual Reports, 1967, p. 20), attention was directed to the minor components of marihuana and to more refined analytical procedures. dl-Cannabichromene (31) was prepared by chloranil dehydrogenation of cannabigerol (32); the pure material was devoid of CNS activity[119]. Cannabichromene was converted photolytically in good yield to cannabicyclol by Crombie et al.[120] whose previously proposed structure (33) was questioned by other workers[121]. The latter group also reported that the reaction of olivetol with citral in pyridine gave cannabichromene and cannabicylol[121]. Cyclizations leading to the THC's can take a different course when the catalyst is altered, thus affording an entry into the iso-cannabinoids. Cannabidiol (34) could be cyclised either to Δ^1- and $\Delta^{1(6)}$-trans-THC (35) or to Δ^8-iso-THC (36); similar examples were reported[122]. Stereospecifically labelled (±)-$\Delta^{1(6)}$-THC-2-axial-^{3}H, suitable for metabolic studies, was prepared[123]. The acid-catalysed condensation of olivetol and pulegone gave

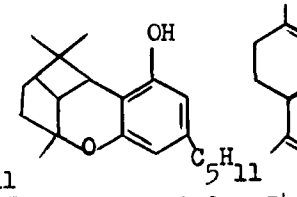
Δ^3-THC (35, Δ^3) together with an equal amount of an isomeric xanthene derivative[124]. A number of THC analogues (35, C_5H_{11} replaced by CH_3) and related xanthenes were prepared[125]. The eight stereoisomers of a biologically active THC congener [35, Δ^3; C_5H_{11} replaced by $CHMeCHMeC_5H_{11}$ were synthesised[126]. Neither a steroidal (37)[127] nor an aza (38)[128] analogue of Δ^3-THC had prominent CNS activities. The phenolic components of hemp plants from different sources as well as the chemical transformations involved on smoking the materials were studied[129]. Newly identified hemp components were cannabigerol monomethyl ether[130a], cannabichromenic acid[130b] and a tetrahydrocannabitriol ester of cannabidiolic acid[131].



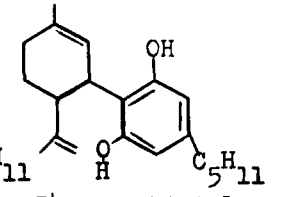
31 cannabichromene



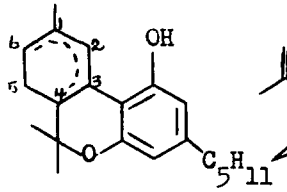
32 cannabigerol



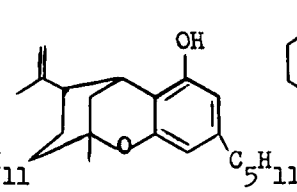
33 cannabicyclol



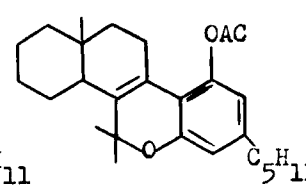
34 cannabidiol



35 THC



36 Δ^8-iso-THC



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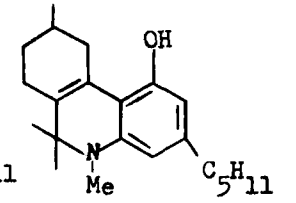


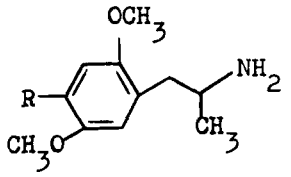
38

A review of mescaline and related compounds, covering chemical and biological aspects of ring-oxygenated phenethylamines, has appeared[132]. Shulgin and co-workers[133], in an important paper, examined structure-activity relationships in a series of alkoxylated phenethyl- and phenylisopropylamines in terms of psychotomimetic potency in humans. Of the 44 compounds examined, 39a was distinctly the most active (80 x mescaline). Possible mechanisms of action of these compounds were discussed and 40 was proposed as a possible endogenous psychotogen. The ethyl homologues of 2,4,5-trimethoxyphenylisopropylamine (39b), a potent hallucinogen, were prepared; compound 39c was claimed to exceed 39b in activity[134].

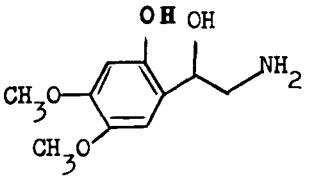
p-Substituted amphetamines were studied in animals using a Sidman avoidance test. The order of activity was Cl and F > CH_3 and in contrast to the p-methoxy compound, the methyl analogue was not a hallucinogen[135]. The ability of substituted amphetamines to release cardiac NE was investigated; compounds with high activity included the 3-CH_3, 4-OH, 3-OCH_3, 4-F and 3,4-$(OH)_2$ derivatives[136]. The cyclopropylamine 41, related structurally to both mescaline and tranylcypromine, elicited behavioural effects in rodents similar to those of the former drug.[137].

Several 3-hydroxyindole derivatives, including 1-methylindican, indoxyl-O-acetate, adrenolutin and adrenochrome were highly active in an animal behavioural test. Removal or blocking the hydroxyl group by

etherification abolished the activity, a finding which lends support to the theory that abnormal metabolic processes could produce such psychotogens in disordered states[138]. Hydroxylation at the 4-position of the indole nucleus could also play a role[139]. Structure-activity relationships (failure of conditioned avoidance in rats) and partition coefficients in a series of tryptamines (42) were investigated[140]. Others have studied the relationship between psychotropic activity and electron-donating ability in substituted indoles and tryptamines[141]. A similar approach was used for a series of piperidinol esters[142]. The central stimulant activities of several benzothiophene derivatives (43) closely resembled those of the corresponding tryptamine isosteres, supporting the hypothesis that the indolic NH group does not significantly interact with tryptamine receptors[143]. The potential for developing new psychotropic agents using this approach is considerable.



39 a, R=CH_3 (DOM)
b, R=OCH_3
c, R=OC_2H_5



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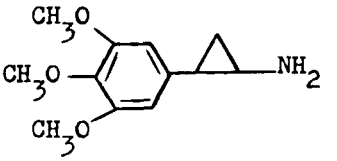
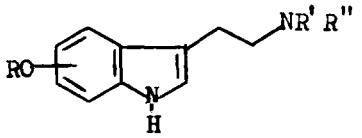


41

In a series of piperidyl glycollates a significant correlation was found between the potency in blocking oxotremorine-induced tremors in mice and in elevating the EEG arousal in cats. The finding is in agreement with the view that the psychotomimetic activity of this class of compounds is mediated through a central cholinergic blockade[144]. The glycollate 44 was reported to possess marked behavioural and anticholinergic properties[145].



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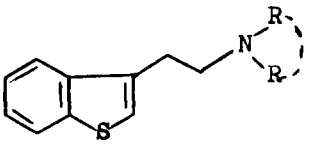

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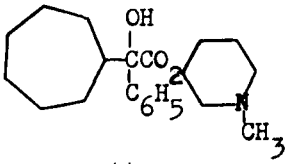
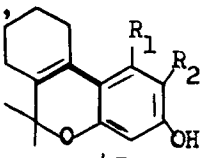


44

Biology - The availability of pure, synthetic Δ^1- and $\Delta^{1(6)}$-THC's (35) has prompted interesting pharmacological studies. Both isomers exhibited pronounced analgesic action in mice and rabbits, using a number of test procedures. The analgesic action of a 20 mg/kg dose (i.p.) was equivalent to that of 10 mg/kg morphine sulfate (s.c.) and a proposal was made that $\Delta^{1(6)}$-THC could be a potential non-addictive analgesic drug[146]. The psychotropic effects could, however, militate against such a use. The two isomers caused complex behavioural patterns in monkeys including apparent visual hallucinations, alteration of avoidance response, panic and stimulation followed by a prolonged depression[147]. These findings are of importance in view of the relatively non-specific behavioural effects of

marihuana observed in other animal species. Rats rendered tolerant to Δ^1-THC and cannabis extract were still sensitive to LSD and mescaline. The results suggest that despite the similarity of symptoms produced in man by the 3 drugs, Δ^1-THC may act by mechanisms different from those of the other two[148]. Cross-tolerance between LSD and mescaline in the rat has been demonstrated[148,149]. It is interesting to note that in man, a sensitisation or "reverse-tolerance" to the effects of marihuana has been noted[150,151]. A pharmacological study of Δ^3-THC analogues (45, R=H,OH; R_2=H, Cl, C_6H_{13} and their aromatized forms indicated that none of the compounds would have psychogenic action in man[152]. Cannabis resin caused teratogenic damage in rats[153].



45

EEG studies of amphetamine and the potent hallucinogenic analogue DOM (STP; 39a) showed that the drugs act by different mechanisms[154]. Other methoxylated amphetamines were found to differ from the unsubstituted drug in their effects on swimming behaviour of rats[155]. The pharmacological actions of 3,4-dimethoxyphenethylamine (DMPEA), a proposed endogenous hallucinogen, were generally similar to those of one of its metabolites, N-acetyl DMPEA. The latter, however, produced maniacal behaviour in cats and may be the actual psychotogenic factor[156,157]. Studies of the dispositon and metabolism of DMPEA were reported[157,158] together with further evidence for its action in the CNS[158]. The mechanism of action of LSD remains unresolved and the current theories were briefly reviewed[159]. Recent work indicated a central mediation through NE[159,160] and/or 5-HT[161].

A stereotyped, bizarre behaviour was produced in rats by known hallucinogens, including the narcotic antagonists cyclazocine and levallorphan. The test may be of use in predicting the hallucinogenic activity of new drugs in man[162]. In a recent paper, Smythies and coworkers[163] discussed problems of methodology and reviewed structure-activity relationships in the substituted phenethyl- and phenylisopropylamines and the tryptamines. Their development of a sophisticated experimental design, based on operant conditioning schedules in the rat, is a valuable contribution to the field.

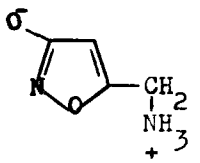
Clinical - Reflecting public anxiety, a great number of articles have appeared in the past year concerning the use and abuse of hallucinogenic drugs. The problem of marihuana was treated in an authoritative British report[150]. Two of the findings are (i) long-term consumption in moderate doses has no harmful physical effects and (ii) the properties of the drug per se do not lead to addiction or to progression to other drugs. The authors agree, however, with the recent conclusion of the AMA Council on Mental Health that it is a "dangerous" drug but in terms of physical harmfulness, it is less so than the opiates, amphetamines, barbiturates or even alcohol. General papers worth noting include those on drug dependence[164a] and abuse[164b-d], with implications for research[164e]; psychiatric insights into the current "drug problem"[165] and a collection of clinical reports on LSD, STP(DOM) and marihuana[166]. The practicability of LSD as a clinical tool as well as the dangers attending its use[167] and possible chromosomal and teratogenic effects[168] were reviewed. Hollister[88b], in a incisive survey of the current status of psychotherapeutic drugs, has referred to LSD and

related agents as 'drugs in search of disorders'.

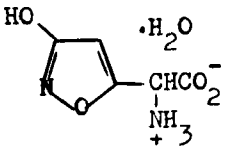
The psychological and physiological effects of marihuana were studied in volunteers in a neutral setting and on a double-blind, placebo-controlled basis. The principal action was that of a mild intoxication with relatively few somatic effects being observed[151]. The clinical effect of Δ^1-THC and an analogue (35, Δ^3; C_5H_{11} replaced by n-C_6H_{13}; Synhexyl) were found to be quite similar, with the latter being more prolonged[169].

The active principles of the Mexican drug ololiuqui, d-lysergic- and d-isolysergic acid amides, were studied in normal subjects. The effects produced were said to differ from those seen with the usual psychotomimetics and to more closely resemble those of scopolamine and ibogaline, i.e. a toxic psychosis with marked autonomic reactions[170]. Recent interest has been shown in the toadstool Amanita muscaria ("fly agaric"). Two of the active principles are the isoxazoles muscimole (46) and ibotenic acid (47), both of which are now available synthetically[118a]. The compounds were studied in animals and man; clinically they produced an intoxication which was not, however, characteristic of a genuine model psychosis[171]. The physiologic, neurophysiologic and psychologic effects of psilocybin (48) on normal subjects were investigated. Marked perceptual, affective and cognitive changes were recorded[172]. It was proposed that this drug or its analogues should replace LSD in experimental psychiatry due to the shorter duration of action and low incidence of side-effects[173].

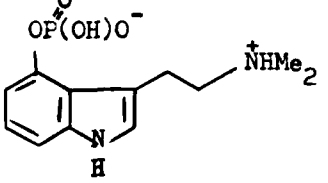
Concern has been expressed over the use of readily-available drugs such as belladonna, stramonium[174] and other "proprietary hallucinogens"[175] which can cause toxic psychoses.



46 muscimole



47 ibotenic acid



48 psilocybin

SUMMARY

Relatively little progress was made in the past year on the development of significantly new or improved antidepressants. Major structural departures from the established drugs gave several agents which fulfilled laboratory criteria for activity but which were later shown to be ineffective in the clinic. Newer approaches to the methods of screening these drugs are required together with a fuller understanding of the biochemical processes they engender.

More encouraging results were seen from work done on the hallucinogens. The important chemical, pharmacological and clinical studies of marihuana are beginning to replace hearsay, folklore and prejudice by experimentally verifiable facts and a proper evaluation of this complex drug should emerge. Similar investigations of other naturally occurring hallucinogens are being undertaken. Structural modifications of compounds resembling the biogenic

amines, such as the phenethylamines and the tryptamines, are bringing about a better understanding of the delusional states and could, hopefully, lead to new classes of psychotherapeutic agents.

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Chapter 3. Sedatives, Hypnotics, Anticonvulsants, Muscle Relaxants, General Anesthetics

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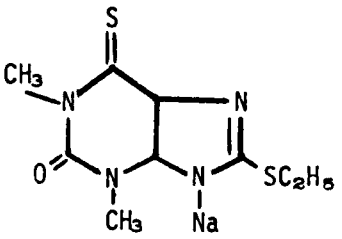
Introduction - Following the format of previous editions of this chapter the discussion of compounds is divided on the basis of established or projected clinical use and to a lesser extent on the basis of pharmacological properties rather than on the basis of chemical structure. An effort was made to exclude those depressants more properly classified as antipsychotics and antianxiety agents which are discussed in Chapter 1, although there is undoubtedly some overlap. Further, an effort was made to scan all of the published literature in the subject therapeutic areas, but only those compounds with more than preliminary pharmacological screening data or representative of new structures were selected for inclusion in the review.

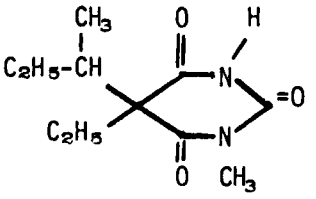
Related to the compounds of these classifications and other CNS agents is the isolation by Riddell and Leonard[1,2] of a high molecular weight material from mammalian brain which caused a loss of righting reflex when injected intravenously or intracerebrally into mice, rats or rabbits. Recovery of the righting reflex was followed by prolonged behavorial depression. The activity was found in the brain and spinal cord of eight mammalian species including man, but is absent from non-nervous tissue.

Sedatives and Hypnotics - The hypnotic properties of the theophylline derivative ETTT (I) in mice, rats, rabbits and dogs has been described[3]. The activity was related to the dose but at active doses dogs experienced respiratory stimulation, vomiting and defecation.

The hypnotic efficiency of N-methylbutisol (II) was evaluated in patients against the provocation to awaken to empty the bladder[4]. The



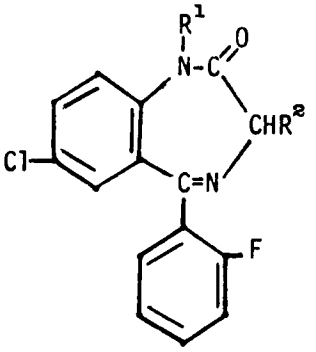
I



II

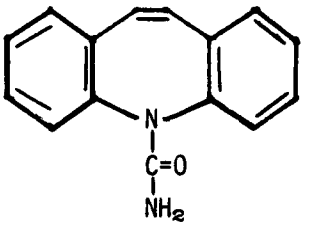
compound was inferior to pentobarbital and the investigator concluded there were no grounds for recommending the addition of this new agent to the presently available range of hypnotics.

A study[5] of the urinary metabolites of the hypnotic, flurazepam hydrochloride, Ro 5-6901 (III), in the dog reveals non-conjugated (IV) and (V), conjugated (IV), (V), (VI) and (VIII) and a phenolic derivative of (VII). Human urine contained only the glucosuronic acid or sulfate conjugate of (VI). The synthesis and a comparison of the pharmacology of flurazepam hydrochloride with that of other 1,4-benzodiazepines has been reported[6], as well as the clinical use of this compound as a hypnotic[7].



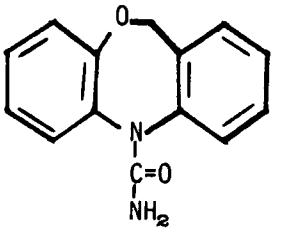
	R^1	R^2
(III)	$-CH_2CH_2N(C_2H_5)_2$	H
(IV)	$-CH_2CH_2NHC_2H_5$	H
(V)	$-CH_2CH_2NH_2$	H
(VI)	$-CH_2CH_2OH$	H
(VII)	-H	H
(VIII)	-H	OH

Anticonvulsants - Carbamazepine (IX), already commercially available in Europe, was cleared by the United States Food and Drug Administration for the indication of relief of pain of trigeminal neuralgia[8,9].

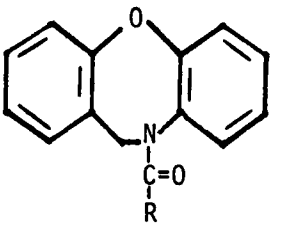


IX

Yale[10] has prepared the carbamazepine analog (X) and found it to be equally active as the prototype in reducing the amplitude of trigeminal or thalamic evoked potential in cats following intravenous administration, but with an apparent longer duration of action and more selective effect on the trigeminal sensory system. In a related series compound XIa was not active while the corresponding semicarbazide (XIb) was an effective anticonvulsant against electroshock in the mouse[11].



X

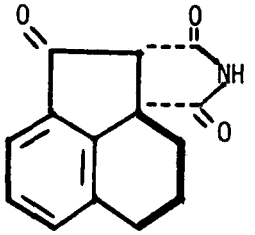


a) R = NH_2
b) R = $NHNH_2$

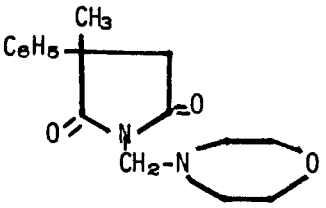
XI

Among the succinimide derivatives CM-6 (XII)[12] was reported to be equipotent with diphenylhydantoin but shorter acting and less toxic in mice challenged with electroshock or pentylenetetrazole. In psychotic patients who received 200 - 1000 mg. of CM-6 per day for a mean of sixteen and a half days, seven of ten became worse and three remained unchanged. It activated the psychotic syndrome and intensified hallucinatory activity. This study[13], together with the report[14] that the compound causes rapid onset of visual disturbances, indicates that CM-6 will not be a useful anticonvulsant.

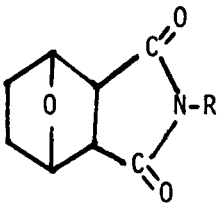
Another succinimide reportedly being used clinically in Hungary is perlepsin (XIII)[15]. A study[16] of sixty-six 7-oxabicyclo-[2.2.1]heptane-2,-3-dicarboximides (XIV) where the N-substituent was varied led to several



XII



XIII



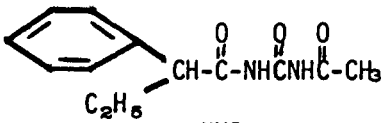
XIV

compounds active as anticonvulsants against electroshock and pentylenetetrazole. The active compounds were limited to those in which R was aryl or aralkyl and these compared favorably in the animal tests to drugs currently used in therapy. The compound having R equal to 2,3-dichlorophenyl showed the greatest overall activity.

The silicone containing compound (XV) protected against electroshock and pentylenetetrazole convulsions at doses lower than the parent phenylacetylurea after oral administration to mice[17]. The compound was devoid of any sedative activity.

Crampol (XVI) is reported to have a better therapeutic index than phenylacetylurea in inhibiting electroshock convulsions in mice[18]. The compound is being studied clinically.

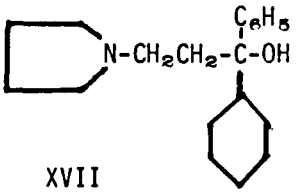
XV



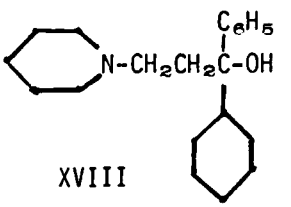
XVI

The antiparkinsonism agents, procyclidine (XVII) and trihexyphenidyl (XVIII) reduced the incidence of epileptic seizures in children resistant to other anticonvulsants[19]. Procyclidine was the more potent. This should

encourage further investigation of this class of atropine-like compounds for anticonvulsant activity.

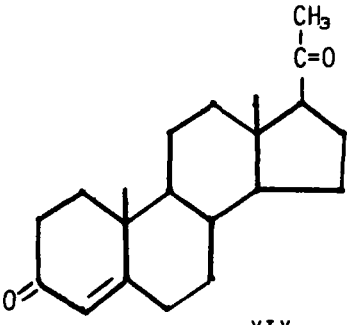


XVII

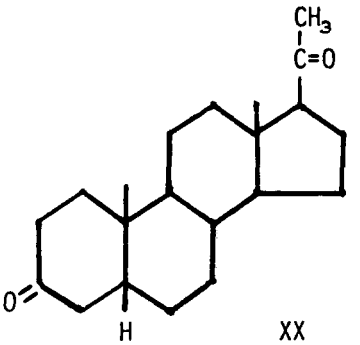


XVIII

The effect of substitution on the activity of some thirty variously substituted 4-pregnen-3,20-diones (XIX) and 5β-pregnanediones (XX) as antagonists of pentylenetetrazole-induced convulsions in the mouse showed



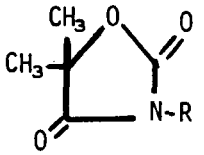
XIX



XX

that addition of functional groups produced compounds with reduced activity compared to the parent compounds[20]. However, changes in structure did result in altered activity so it was concluded that it is possible that among steroid compounds can be found those which possess much greater anticonvulsant activity.

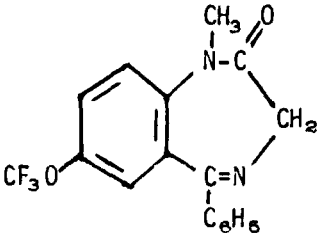
DMO (XXIa), a metabolite of trimethadione, TMO (XXIb), was reported to have similar anticonvulsant properties in rats and mice but it is weaker and cannot account for the total activity of the parent drug[21].



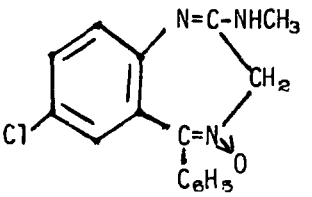
a) R = H
b) R = CH_3

XXI

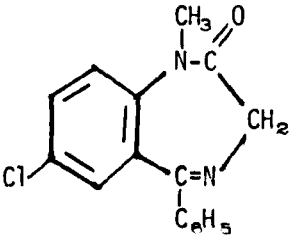
Compound (XXII) resulted from a study of several 7-trifluoromethoxy and 7-trifluoromethylthio derivatives of 1,4-benzodiazepines[22]. When tested for its ability to induce ataxia, to decrease locomotor activity and to afford protection against electroshock and strychnine-induced convulsions in mice, it had activity, potency and acute toxicity similar to those of chlordiazepoxide (XXIII). The utility of XXIII and diazepam (XXIV) as useful anticonvulsants has been demonstrated[23,24,25,26].



XXII



XXIII

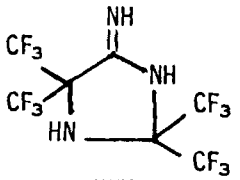


XXIV

Central Muscle Relaxants - There appeared during the year an excellent review of the synthetic centrally acting skeletal muscle relaxants which covers the literature through most of 1966[27]. As the authors, Donahoe and Kimura, point out, there continues to be a need for effective chemical compounds which relieve painful skeletal muscle spasms without producing muscular weakness or incapacitating sedative or hypnotic effects. Since most of the clinically useful compounds fall within a few structural classes there is a need for more basic studies involving molecular alteration for evaluation as central muscle relaxants.

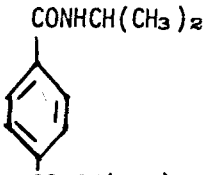
Although it has been clinically available for a number of years, the United States Food and Drug Administration in early 1968 allowed labeling changes for diazepam (XXIV) to allow for new indications of use for the relief of muscle spasm and adjunctive use in convulsive disorders[28]. It was reported[29] that in cats the brain stem reticular system is the major locus of central nervous system depressant action of this centrally acting skeletal muscle relaxant.

EXP-338 (XXV) causes skeletal muscle relaxation in rats and dogs at 1-2 mg. per kg. orally[30]. The activity of this compound in various preparations and its relatively low toxicity indicate a promising potential for EXP-338. In a quantitative non-blind evaluation of the compound in fifteen multiple sclerotic patients, Levine et al.[31] observed reduced muscle hypertonia of an average of 62% in half the patients. The most common side effects were muscle weakness, insomnia and paranoid reactions.

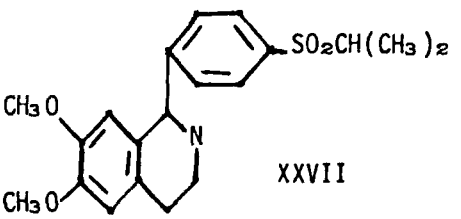


XXV

Compounds (XXVI) and (XXVII) were reported to have about the same order of muscle relaxing effect in the mouse and cat as chloromezanone[32]. Esterification of mephenesin with

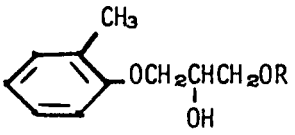


XXVI



XXVII

unsubstituted or the o-hydroxy, p-chloro or p-fluorophenoxyacetic acid (XXVIIIa) increased the duration of muscle relaxant activity in mice from 1.5 to 3 times over that of mephenesin carbamate (XXVIIIb), while lowering the intensity of action[33].

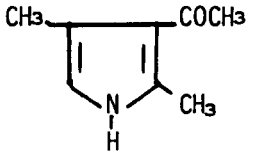


XXVIII

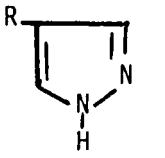
a) R = $-\overset{\overset{O}{\|}}{C}CH_2O-$

b) R = $-\overset{\overset{O}{\|}}{C}-NH_2$

Moffett[34] reported that the pyrrole (XXIX) showed enough promise in animals that it was studied in man as a muscle relaxant and tranquilizer but side effects precluded doses large enough to observe CNS effects. The related series of pyrazoles (XXX) produced muscle relaxant effects in mice[35].



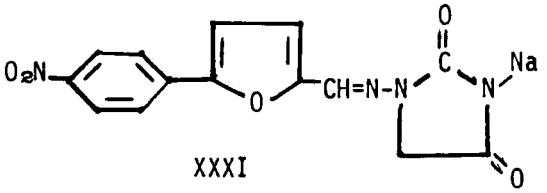
XXIX



R = C_2 - C_4

XXX

A spectrophotofluorometric procedure for the determination of the potent muscle relaxant, dantrolene sodium (XXXI), in blood was reported[36].



XXXI

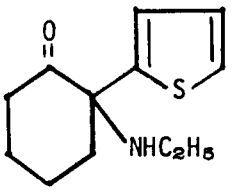
The synthesis and pharmacology of this and related compounds have appeared previously[37].

General Anesthetics - Again during 1968 there was little published which could be considered an appreciable advance in this area of medicinal chemistry.

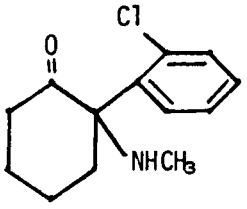
Lear[38] has reviewed three chemically unrelated series of compounds used for intravenous anesthesia, that is, derivatives of cyclohexylamines such as ketamine (XXXIII), phenoxyacetamides such as propanidid, and γ-hydroxybutyric acid. Comparative pharmacology and advantages and disadvantages are listed.

The usefulness of γ-hydroxybutyric acid as an agent for general anesthesia continues[39,40]. This compound is the metabolite of 1,4-butanediol, and the effects of the latter are antagonized by the antagonist of γ-hydroxybutyrate, β-hydroxybutyrate. This evidence is taken as support for the view that γ-hydroxybutyrate is the active metabolite involved in the production of anesthesia by 1,4-butanediol[41].

Chen and co-workers describe surgical anesthesia in the cat[42] and rabbit[43] using CI-634 (XXXII), an analog of ketamine, CI-581 (XXXIII). The comparative pharmacology of the two compounds has been described[44].



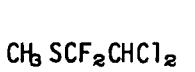
XXXII



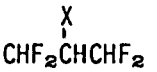
XXXIII

Methioflurane (XXXIV), a new volatile anesthetic has been used intravenously in dogs and monkeys where it proved to be a safe and effective anesthetic[45]. Resuscitation of the animals was made easy because of the ability of the lungs to excrete the drug. The oxygen analog has been similarly used in man and animals[46].

Among the volatile anesthetics the halogenated propanes (XXXV) are claimed to be useful, non-flammable inhalation anesthetics in mice with a low order of heptatotoxicity[47].

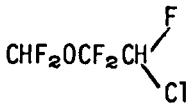


XXXIV



X = Cl, Br

XXXV



XXXVI

Compound 347 (XXXVI) produced satisfactory anesthesia in dogs according to McDowell et al.[48] but was not compatible with epinephrine and produced poor analgesia. Results obtained by Dobkin et al.[49] when the drug was given with nitrous oxide-oxygen to 100 patients indicated that it deserved further clinical evaluation.

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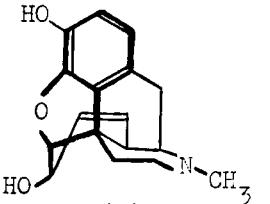
Chapter 4. Analgesic Agents

J.F. Cavalla, John Wyeth & Brother, Taplow, Maidenhead, Berkshire, U.K.

There has been no dramatic happening in the field of analgesics in 1968, although continued progress has been made towards the eventual introduction of an orally active non-addictive strong analgesic. Important work has been reported regarding the pharmacological and clinical evaluation of analgesics especially with regard to the assessment of narcotic antagonists. Comparison of the novel structures which have been prepared and found possessed of analgesic activity indicates that the rational design of a potent morphinomimetic or morphine antagonist is still far from predictable. No real progress has been made in the work on the mode of action of analgesics.

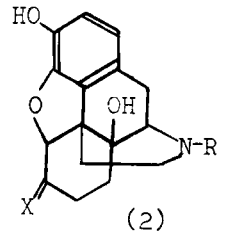
The report of the proceedings of an international symposium on pain was published.[1] This includes work from over 40 contributors on the laboratory and clinical evaluation of analgesics with reviews of currently favored concepts of the mechanism of the perception of pain and its blockade. A more conventional account of those morphine-level analgesics known to 1965 is given by Janssen and van der Eycken.[2] In a critical review,[3] Lim presents that recent work which has been reported on the site of action of analgesics and the nature of the pain receptors



(1)

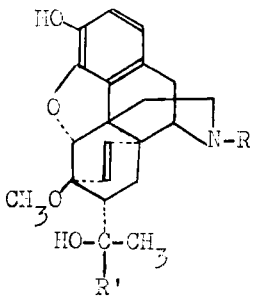
I Strong Analgesics A. Morphine-like compounds - The absolute configuration of morphine has been elucidated[4] and is shown (1). The methoxymethyl ether of morphine[5] proved less active than the parent while the essential inactivity of morphine N-oxide has been confirmed.[6]

The potential value of the pure antagonist naloxone (2, $R=CH_2CH=CH_2$; X=O)[7] and its derivatives continues to excite attention. Clinically, naloxone has been shown to be useful in counteracting heroin dependence[8] as well as antagonizing the miotic, the respiratory depressant and the subjective effects of cyclazocine,[9] and the respiratory effects of pentazocine.[9a] The fact that naloxone will antagonise other narcotic antagonists renders more complex the relationship between narcotics, their antagonists and their mutual interaction. These are the first examples of the antagonism of one antagonist by another but, no doubt, others will follow. The related dimethylallyl compound, EN 1620A, nalmexone (2, $R=CH_2CH=C(CH_3)_2$; X=O), and its reduction product (2, $R=CH_2CH=C(CH_3)_2$; X=H,OH) are the subject of a patent[10] while the reduced cyclobutylmethyl compound, EN 2234A, nalbuphine (2, $R=CH_2CH(CH_2)_3$; X=H,OH), is claimed[11] to be a morphine antagonist showing central analgesia (hot plate) and possessed of low toxicity.



(2)

Further examples of analgesics derived from thebaine (see ref. 7, p. 37) have been reported. The allyl derivative (3, R=$CH_2CH=CH_2$; R' = $CH_2CH_2CH_3$) is stated[12] to be a potent morphine-like analgesic with no nalorphine properties, yet possessing a good separation of analgesic from respiratory depressant properties. A compound (4; M 5050) having a



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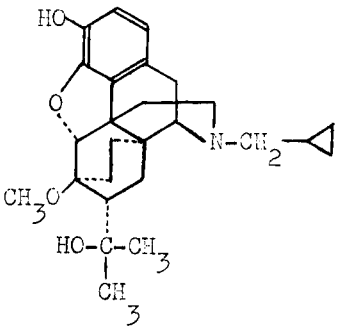

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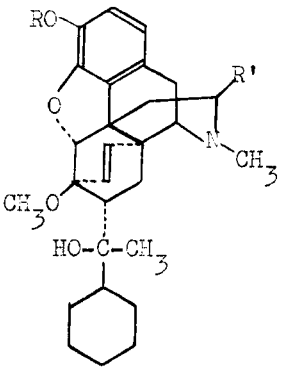
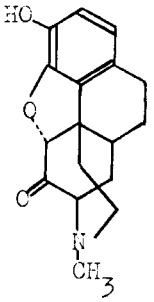


(5)

reduced C_7-C_8 bridging function shows[13] potent naloxone-like activity with strong antagonist but no analgesic effects (bradykinin and phenylbenzoquinone). The cyclohexyl carbinol (5,R=R'=H), M 125, produces its biochemical and pharmacological effects by similar mechanisms to morphine, although its activity is 1,000 times greater.[14] A related ring methylated compound (5,R=R'=CH_3), M 6420,[15] is a very weak analgesic (0.5 x codeine) but exhibits a potent (12 x codeine) antitussive effect.

Three rearrangement products of morphine: (6)[16], (7,X=O and H_2)[17] and (8)[18] have been prepared. All were of low activity (<0.5 x codeine).



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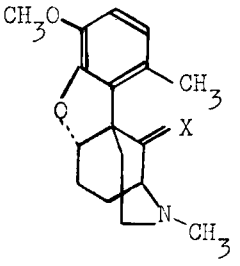

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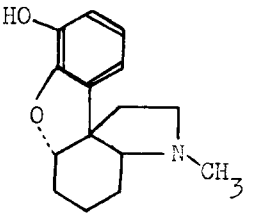
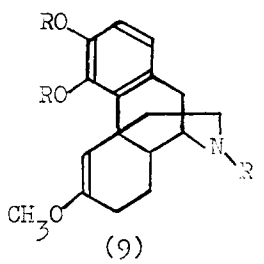


(8)

Opening the furan ring in morphine gave[19] compounds (9) equianalgesic with morphine but claimed to be antagonists.



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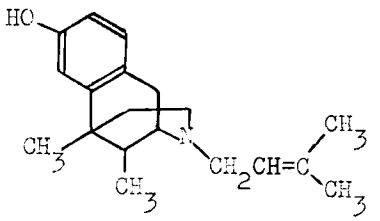

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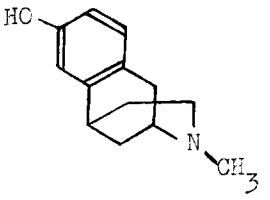

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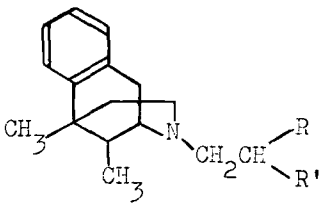

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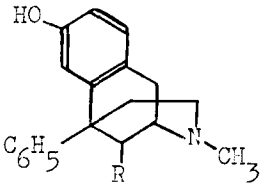


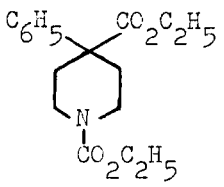
(13)

B. Benzomorphans - Pentazocine (10) has been shown[20] to have oral activity of an order one quarter of that found parenterally. In a parenteral comparison, respiratory depression is stated[21] to equal that shown by morphine. The absence of addictive liability with pentazocine has been questioned.[22]

Variations of the benzomorphan analgesics which have been reported include the bis-desmethyl compound (11)[23] and compounds with complex nitrogen substituents (12,R=OH and Br ; R'=H, CH_3 and C_6H_5),[24] none of which shows marked activity. The 5-phenyl derivatives (13,R=H) are the subject of a patent.[25] The levo isomer of the phenyl substituted derivative (13,R=CH_3) shows morphine level analgesia[26] with no capacity to substitute for morphine in addicted rats; on the other hand, the dextro isomer is inactive as an analgesic yet suppresses the abstinence syndrome. A ready synthesis of benzomorphans by cyclization of 2-substituted benzyl-1,3-dimethyl-4-piperidinols has been patented.[27]

C. Meperidine-related Compounds - The earlier report that meperidine N-oxide was equiactive with meperidine[28] has been supplemented by the discovery[29] that the urethane of nor-meperidine (14) is also analgesic with a low physical dependence capacity. The possibility that both the N-oxide and the carbethoxy group are latentiating groups and that the active moiety in both cases is nor-meperidine, released by metabolic attack at the site of action, should encourage the preparation of many related structures.

The adamantyl ester of meperidine[30] is claimed to be more potent than meperidine but with no other advantage. Similarly the N-allyl and



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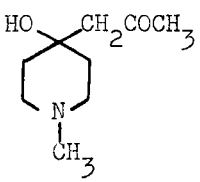

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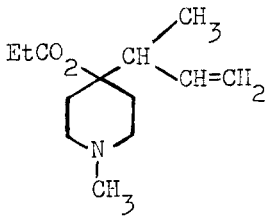
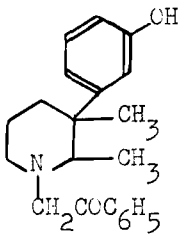


(16)

dimethylallyl derivatives of meperidine and the prodines[31] present no advantageous features. The more diverse structures (15)[32] and (16)[33] are claimed to be analgesic-antitussives.



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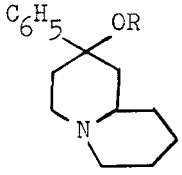

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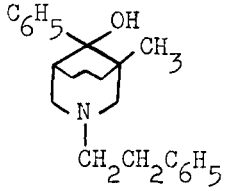
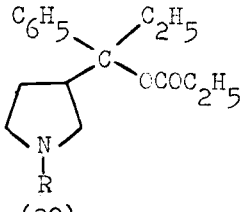


(19)

The detailed pharmacology of the 1,2,3,3-tetrasubstituted piperidine, TA 306 (17) has been published.[34] The compound does not relieve the withdrawal syndrome in addicted monkeys; in mice and rats it shows meperidine-level analgesia and in dogs codeine-level antitussive action. The fused ring systems (18,R=H and acyl)[35] and (19)[36] are active analgesics.



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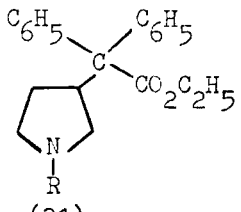

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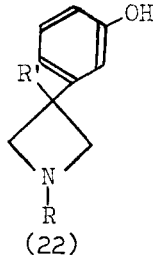
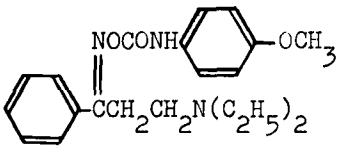


(22)

The pyrrolidines, AHR 1767 (20,R=CH_3),[37] (20,R=$CH_2CH_2CH_2C_6H_5$)[38] and (21)[39] have propoxyphene-level activity. The azetidines (22)[40] possess meperidine-level analgesic activity comparable with that of their previously prepared non-addicting pyrrolidine analogues (see ref. 7, p. 39).

D. Miscellaneous - Various compounds reported this year are claimed to possess analgesic potency equal to or better than codeine. The basic propiophenone oxime, USVP E 142 (23) has morphine-level activity[41] with no respiratory depression, suppression of withdrawal syndrome capacity or nalorphine interaction. Related compounds (24), of similar potency, were reported at the Spring A.C.S. meeting[42]. The pharmacology of aletamine

(25,R=H), a compound claimed not to suppress the withdrawal syndrome, has been published[43] along with the chemistry of a series of analogues (25),[44]



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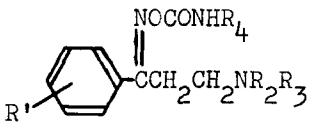

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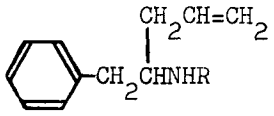
The basic pyrroles (26)[45] are analgesic at 10 mg/kg i.p. The phenampromid analogue, Propiram, Bayer 4503 (27,R=H) is a morphine antagonist;[46] sub-



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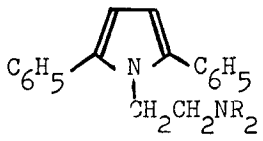

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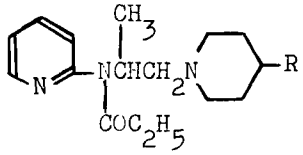
stitution on the piperidine ring (27,R=C_6H_5) gives more active, but morphine-like, compounds.



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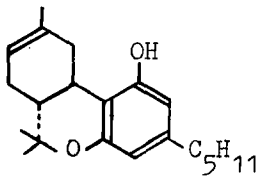

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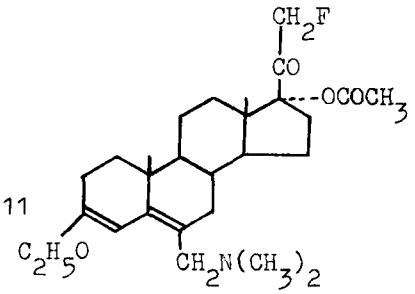

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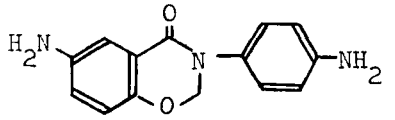
$\Delta^{1,6}$Tetrahydrocannabinol (28) has been shown[47] to possess about one half the activity of morphine in mice and rabbits using a variety of tests. The nature of the analgesia is said to differ from that of morphine and it is speculated that the compound will induce habituation rather than addiction. The pharmacology of the steroid, SC 17599 (29) (cf. ref. 7, p. 38) has been reported:[48] It is less active than morphine in the mouse writhing and hot plate test and in the rat tail flick, but would appear to demonstrate true analgesia.

II Weak Analgesics - For complete coverage of this area Chapter 19 on anti-inflammatory drugs should be consulted.

A. Salicylates - The prominence[49] given to the hazards associated with aspirin and phenacetin lends encouragement to the search for superior compounds. The n-hexylcarbonate of salicylic acid[50] is claimed to have activity equal to that of aspirin but with no ulcerogenicity. 2-Hydroxy-3-isobutyl-6-methylbenzoic acid is superior[51] to aspirin as an analgesic.

The cyclic salicylamide (30)[52] performs slightly better than aspirin in the mouse writhing and hot plate tests.[53] Aspirin itself has been found



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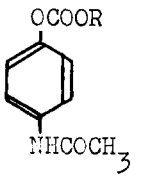
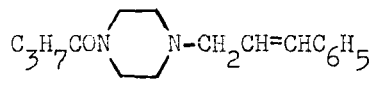


(31)

to be rapidly hydrolysed to salicylic acid in blood serum, implying that any action of the drug is due to free acid, the acetyl group on the phenol merely allowing of good absorption.

B. Aniline Derivatives - Several paracetamol analogues (31, R=C_6H_5, $CH(CH_3)_2$ and CH_2CCl_3 have been made,[54,55,56] some with marginal improvement over the model. m-Trifluoromethylacetanilide[57] is superior to aspirin, showing one-eighth the activity of codeine with no evidence of methemoglobinemia.

C. Heterocyclic Compounds - The presence of analgesic activity in substituted piperazines is vouchsafed in several reports. The cinnamyl derivatives (32)[58] and (33)[59] are more active than aminopyrine, while the diaza-



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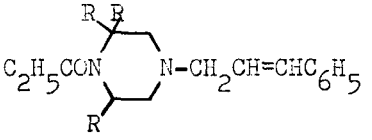

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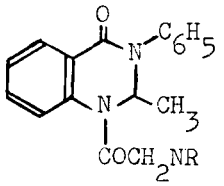
bicyclooctane (34, R=CH_3), in a double-blind clinical trial,[60] proved less active than codeine-aspirin mixture as an analgesic but equiactive with codeine alone as an antitussive. Related compounds (34, R=$CH_2CH_2C_6H_5$)[61] and (34, R=$CH_2CH=CHC_6H_5$)[62] show near-codeine potency in small animals.

Aminopyrine-level analgesia associated with anti-inflammatory activity was found in the tetrahydroquinazolines (35, R=$(C_2H_5)_2$ or $-(CH_2)_5-$)[63] and the pyridazinone (36),[64] while the imidazolines (37, R=lower alkyl or halogen) showed[65] non-specific activity in the writhing test. Analogues of the analeptic, bemegride (38, $R_1=C_2H_5$, $R_2=CH_3$, $R_3=H$) have been found[66] to possess analgesic/anti-inflammatory properties, the best of which (38, $R_1=R_3=CH_3$, $R_2=H$) has many times the potency of aspirin.

D. Miscellaneous - The basic indene (39)[67] was more active than codeine in the writhing test but inactive in the Haffner tail clip test, while the fundamental morphine fragment (40)[68] was completely devoid of activity. In double-blind clinical trials, indomethacin has been found[69] to lack analgesic activity whereas mefenamic acid[70] possessed it. Also clinically, a mefenamic acid analogue, CBA-93626, Sch-10304, 2-(3-chloro-2-methylanilino) nicotinic acid, is claimed[71] to be equivalent to codeine orally with no side effects.



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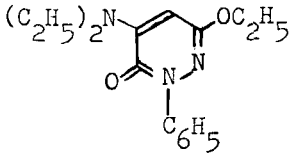

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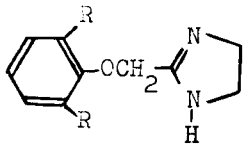

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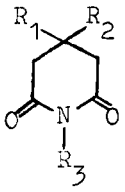

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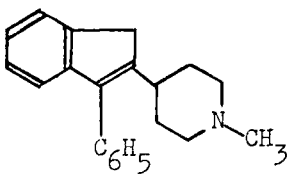

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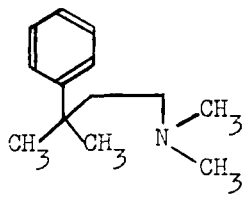

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III Conformation of Strong Analgesics - Papers from both Casy[72] and Portoghese[73] show equivalence of conformation in the active enantiomers of piperidine, diphenylpropylamine, benzomorphan and morphine analgesics. Portoghese,[73a] using 2,5-methenobridged pethidine, brilliantly demonstrates that failure to conform to these configurational requirements does not result in loss of intrinsic analgesic activity. Rather, activity is retained but is not made manifest to the same degree in vivo owing mainly to poorer absorption and transport of the compound to the brain. This finding prejudices a servile reliance on drug receptor interaction for a rationalization of analgesic activity - structure relationships.

IV Pharmacology - With the need to evaluate potentially analgesic narcotic antagonists in the laboratory, more emphasis is being placed on the writhing test. The lack of specificity of this procedure is exemplified by the finding[74] that antidepressants show up better than codeine in the test. Nevertheless, Lasagna[75] has used the method with some success in attempting to demonstrate tolerance to narcotics in mice. Various modifications of the test have been examined such as using a double injection of the nociceptive agent,[76] using arachidonic acid as a writhing agent[77] and extending the test to rats where, using bradykinin, a novel head rotation capable of being relieved by narcotics and narcotic antagonists has been observed, (ref. 1, p. 218). The perennial question of the reliability of this test has been substantially answered by the finding that naloxone, inactive in man, is inactive in the test.[78] More recently, papers by Harris[79] and Collier[80] demonstrate convincingly the good correlation between the human analgesic action of narcotics and narcotic antagonists and their capacity to relieve (in the first case) phenylquinone and (in the second case) acetylcholine induced writhing in mice. It would appear that once analgesic action has been demonstrated in a structural type, either clinically or predictively in animals using an antinociceptive test based on

pressure, heat or electric shock, the writhing test can afford a rapid means of evaluating a series. Furthermore, the test remains the sole means of picking up narcotic antagonists known to be analgesic in man.

V Clinical - The difficulty inherent in attempting to evaluate the action of analgesics in healthy volunteers is discussed by Beecher and others (ref. 1, pp. 201, 223 and 119) with the finding that the optimum method is that based on ischemic arm pain.[81] More recently pain inflicted by pressure on the soft tissue in front of the Achilles tendon[82] and that caused by pressure of a sharp needle point[83] have been claimed to give reproducible and statistically significant results in evaluating aspirin-level analgesics.

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Section II - Pharmacodynamic agents

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Chapter 5. Antihypertensive Drugs

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An unusually large proportion of the research reported during 1968 concerned mechanisms of action of compounds previously reported to be clinically effective. A number of new chemical structures exhibiting hypotensive or antihypertensive activity in animals were discovered and preliminary data in humans reported for some; but potentially major, new advances in the treatment of hypertension were not evident.

Several reviews of antihypertensives and clinical comparisons of the newer compounds appeared.[1-4]

ST 155 (Catapres ®) - Numerous studies have indicated a complex mechanism of action with a centrally mediated reduction of sympathetic outflow to the heart and peripheral vessels as a major component. Evidence comes from cross circulation experiments in dogs;[5] lack of hypotensive effect in spinal cats[6] and pithed rats;[7] activity in debuffered dogs;[8] and reduced discharge in splanchnic and cardiac nerves.[9] Responses to norepinephrine and nerve stimulation are still present.[10] The bradycardia caused by ST 155 is not entirely of central origin since it is detectable after vagotomy and spinal section in dogs.[11] The initial pressor rise is due to α-adrenergic stimulation.[12] The direct cause of the hypotensive effect may depend on the dose. Low doses may cause peripheral vasodilation only, while higher doses also decrease cardiac output. Excessive doses reduce cardiac output without changing peripheral resistance.[13]

New clinical studies have confirmed previous reports of effective antihypertensive action with a low incidence of side effects and no impairment of renal hemodynamics.[14] In cases of poor control with ST 155 alone, combination with methyldopa or guanethidine gave good results without orthostatic or renal problems.[15]

Methyldopa - Strong evidence for a central mechanism of action was reported.[16] Pretreatment with RO-4-4602, (N'-DL-seryl-N-2,3,4-trihydroxybenzylhydrazine) a central and peripheral decarboxylase inhibitor, completely inhibited the

reduction of blood pressure by methyldopa in renal hypertensive rats. Accumulation of α-methyldopamine was inhibited 100% in heart and 75% in brain. Analogous treatment with 3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methylpropionic acid, a decarboxylase inhibitor which does not enter the brain, did not prevent the hypotensive effect and inhibited α-methyldopamine accumulation only in heart.

Infusion of methyldopa into the vertebral artery of cats reduced blood pressure and lowered brain norepinephrine. The same effect on brain amines was seen after i.v. infusion but blood pressure was not changed.[17]

It was demonstrated that in patients taking methyldopa, α-methylnorepinephrine is formed, stored and released as a "false transmitter" as in animals.[18] Tests of adrenergic function in rats at the time of maximum blood pressure reduction failed to show sufficient impairment to account for the hypotension. However, the false transmitter principle may still apply to the action on central neurons.[19]

Studies of the effect of methyldopa in patients with impaired renal function[20] confirmed that renal blood flow and glomerular filtration rate were maintained regardless of posture, and suggested a mechanism of action other than some form of sympathetic blockade.[21]

The occurrence of a positive Coombs test in some patients receiving methyldopa has been further investigated. In rare instances this has been associated with the development of an autoimmune hemolytic process which has been reversible on discontinuance of the drug and/or administration of steroids.[22-27]

Guanethidine-like Compounds - Structure activity relationships for a series of branched alkyl guanidines were reported. t-Octylguanidine was found to show fewer side effects and less toxicity clinically than guanethidine, but its hypotensive effect was not as good or consistent as the latter.[28]

Cyclazenin (1-(2-guanidinoethyl)-1,2,3,6-tetrahydro-4-picoline) was clinically effective in some cases where other agents failed.[29] However persistent side effects--parotid pain and orthostatic hypotension in particular--may limit its usefulness.[30] In combination with methyldopa efficacy was increased and side effects reduced.[31]

Propranolol - The mechanism of the potent hypotensive action[32] of propranolol was studied. Frequently high doses were required and the onset of effect was slow, but problems

with postural and exercise hypotension were absent.[33] Reduction of both systolic and diastolic pressure, slowed heart rate, reduced cardiac output, and increased peripheral resistance were found. Intravenous infusion in either normotensive or hypertensive patients caused about 20% reduction of cardiac output but no lowering of blood pressure.[34] Gradual resetting of the baroreceptors was proposed as the mechanism.

Hydralazine - A review of the recent literature on hydralazine and dihydralazine favors a direct spasmolytic effect on smooth muscle as the mode of action.[35]

Peptides - Investigation of a series of synthetic analogs of caerulein (I) indicated that their hypotensive and secretory effects are separable.[36]

SO_3H
|
Pyr-Gln-Asp-Tyr-Thr-Gly-Trp-Met-Asp-Ph-NH_2 I

The sulfated tyrosine residue and the threonine (or replacement by methionine) are needed for good hypotensive effect. The first three amino acids can be removed without major reduction in activity. Caerulein's effect on blood pressure varies widely among animal species.

Renin-Angiotensin - A substance which inhibits the action of renin (but not angiotensin) has been isolated from kidney, and its presence detected in the blood, kidneys, and liver of rat, dog, hog and man.[37] It reduced the blood pressure of both acute and chronic Goldblatt hypertensive rats. The structure is postulated to be II, with the base differing in some unknown way from ethanolamine or threonine.[38] In vitro studies indicated that the stearoyl group must be removed to produce the active inhibitor.

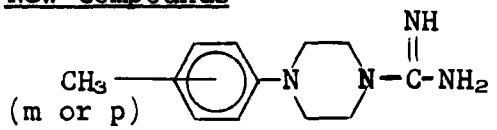
CH_2-O-Arachadonoyl
|
CH-O-Stearoyl II
|
CH_2-O-PO_3-base

False Transmitters - In renal hypertensive rats α-methylnorepinephrine caused a prolonged reduction in blood pressure and a briefer tachycardia. Pronethalol blocked the effect on the heart but not that on blood pressure. Heart norepinephrine was reduced, but not that in brain.[39]

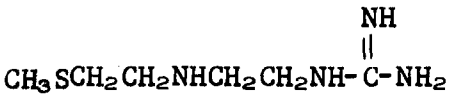
The blood pressure of renal hypertensive (but not normotensive) rats was reduced by 3,5-dihydroxy-4-methoxyphenylalanine.[40] Norepinephrine levels in heart and brain were reduced and 3,5-dihydroxy-4-methoxyphenethylamine was present.[41] Both effects were prevented by the decarboxylase in-

hibitor RO-4-4602. The response to exogenous norepinephrine was not affected.

New Compounds -



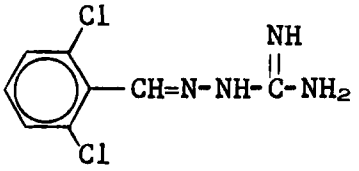
III



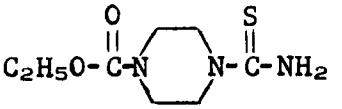
IV

Blood pressure was lowered in rats and cats by III[42] without affecting the pressor response to norepinephrine. Response to tyramine was inhibited.

The guanidine IV showed a guanethidine type of hypotensive action in hypertensive dogs.[43] Blood pressure could be controlled without emesis and diarrhea in the dogs--unlike guanethidine.



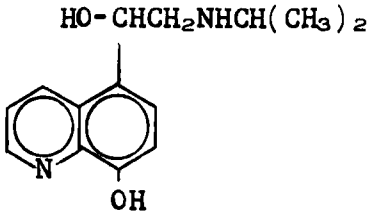
V



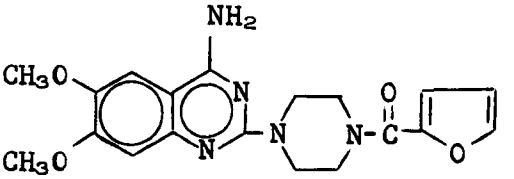
VI

Intravenously in cats, V (SD-15468) caused a brief rise in blood pressure followed by hypotension of long duration during which responses to norepinephrine were potentiated and those to carotid occlusion were reduced. Heart rate was slowed. It was concluded that negative chronotropic and inotropic effects predominated over an α-adrenergic stimulation to produce hypotension.[44]

Of a series of 58 piperazines being investigated for CNS effects, VI was found to have a strong hypotensive effect in anesthetized cats.[45]



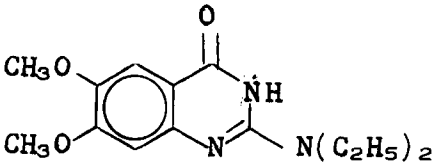
VII



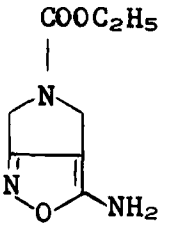
VIII

Quinterenol (VII) represents a new class of β-adrenergic stimulants. It was of primary interest as a bronchodilator, but it decreased mean arterial blood pressure and peripheral resistance in hypertensive dogs, while cardiac output, heart rate, and stroke volume increased.[46]

Prazosin (VIII) and three other diaminoquinazolines lowered systolic blood pressure in hypertensive dogs-- Prazosin at doses as low as 20 γ/kg., p.o. In cats, the pressor effect of epinephrine, but not that of angiotensinamide, was reduced or reversed. The compounds appear to inhibit peripherial sympathetic activity but do not have conventional ganglionic or α-receptor blocking action.[47] Prazosin was effective clinically at oral doses of 3-21 mg., and reduced standing more than supine blood pressure with a short duration of action.[48]



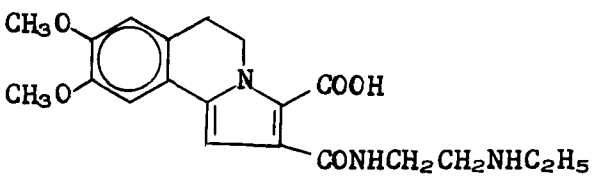
IX



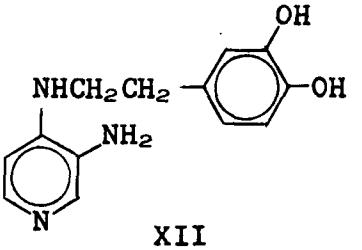
X

Of 54 2-aminoquinazolones tested in Goldblatt hypertensive dogs IX was the most active. No ganglionic blocking activity or lowering of cardiac output was seen. Preliminary clinical tests suggested activity in hypertensives.[49]

Compound X and its N-acetyl (CL-62375) and propionyl derivatives reduced blood pressure in rats but gave erratic results in hypertensive dogs. The compounds inhibited tyrosine hydroxylase and catechol amine binding after repeated doses.[50]



XI

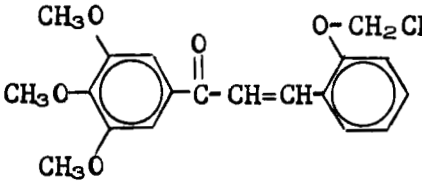


XII

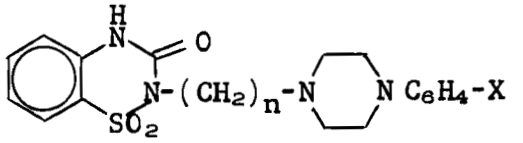
Femoral arterial pressure and peripheral and coronary resistance in dogs were reduced by XI, while coronary flow, cardiac output and contractile force increased. The proper-

ties of XI were unique among the 32 indoloisoquinolines tested.[51]

Blood pressure in cats was reduced by i.v. administration of XII. It appeared to block both α and β receptors with a stronger effect on the latter. Among 64 other 3-aminopyridine congeners tested, only the 3-aminoquinoline analog of XII showed significant hypotensive activity.[52]



XIII

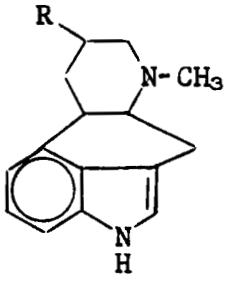


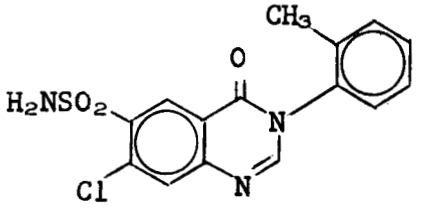
n = 3-5
X = H, m or p-F, m-CF_3

XIV

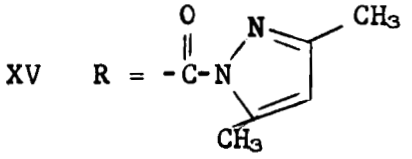
In rats and dogs blood pressure (mainly diastolic) was lowered for up to 8 hrs. by XIII. The carotid occlusion response, and responses to norepinephrine and angiotensin in isolated aorta were blocked. In rabbits, Na^+ and K^+ levels were decreased in serum and increased in aortic tissue--in contrast to effects of other hypotensives tested.[53]

A series of compounds of general structure XIV showed both oral and i.v. activity in reducing blood pressure of normotensive dogs, apparently by α-receptor blockade.[54]





XVII



XV R =

XVI R = $CH_2NH\text{-}CO_2(CH_2)_3CH_3$

A chemically unique lysergic acid congener (XV, PIA) produced sustained lowering of blood pressure in hypertensive dogs and normotensive cats.[55] The mechanism appeared to be α-blockade which was more easily reversible than that of dibenamine. Compound XVI was found to be a strong adrenolytic devoid of oxytocic activity.[56]

Several papers reported the clinical antihypertensive efficacy of a combination of dihydroergocristine, clopamide, and reserpine. Instances of hypokalemia and orthostatic hypotension were rare.[57]

Zaroxolyn (XVII), a thiazide-like diuretic, was hypotensive in DOCA hypertensive rats but did not affect normal blood pressure. It would also prevent the development of hypertension by DOCA administration.[58]

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Chapter 6. Agents Affecting Gastrointestinal Functions

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Digestive diseases include disorders of the stomach, intestine, biliary passages, liver and pancreas, and although there have been many physiological studies concerned with the secretory and motor functions of all of these organs, drug research has been directed almost exclusively to only one aspect, i.e., the treatment of peptic ulcer disease. Extending previous reviews in this series, the present report discusses the developments published in this field during the past two years.[1,2]

Reviews on the chemistry of the gastrointestinal hormones,[3,4] the physiology of histamine,[5,6] gastrin[7,8] and serotonin,[9] the role of gastric proteases and inhibitors,[10,11] and the clinical aspects of peptic ulcer disease[12] were published in 1968. These, along with the proceedings of a symposium on the mechanism and control of gastric secretion,[13] provide a wealth of information and represent excellent summaries for background reading.

Gastrointestinal Hormones

Through the use of new isolation and purification techniques, considerable progress has been made in recent years in the chemistry of the hormonal substances occurring in the mucosa of various parts of the gastrointestinal tract.[4,14] The structures of the gastrins and of secretin have been elucidated and confirmed by synthesis.[15,16] Cholecystokinin (CCK) and pancreozymin (PZ), long considered to be separate entities, have been reported recently to be a single polypeptide containing 33 amino acids.[4,17] The partial structure has been determined and the C-terminal pentapeptide sequence found to be identical with that of gastrin. It is interesting that CCK-PZ bears a close structural resemblance to the decapeptide caerulein, which Erspamer and co-workers have isolated from the skin of the Australian amphibian *Hyla caerulea*;[18,19] the C-terminal octapeptides are identical, except for an exchange of threonine in caerulein by methionine. Moreover, both caerulein and the octapeptide of CCK-PZ elicit many of the biological actions of gastrin and CCK-PZ with considerably greater potency.[20]

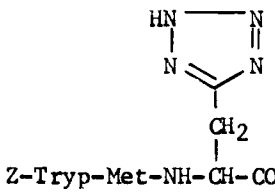
Caerulein Pyr-Gln-Asp-Tyr(SO_3H)-Thr-Gly-Trp-Met-Asp-Phe-NH_2
1 2 3 4 5 6 7 8 9 10

Various biological activities of gastrin, as well as the mechanisms

by which it is released, have been reviewed by Grossman.[21] Endogenously released gastrin stimulates not only gastric acid and pepsin secretion but also pancreatic flow, bicarbonate and enzyme secretion, and hepatic biliary flow. All of these activities, as well as gastric mucosal release of intrinsic factor required for vitamin B_{12} absorption,[22,23] are presumably physiological actions. The development of a sensitive radioiodine immunoassay system, utilizing antibodies to human gastrin I,[24] has allowed the measurement of gastrin levels in physiological fluids and the identification of the gastrin-containing cell in the antral mucosa.[25]

Human Gastrin I Pyr-Gly-Pro-Try-Leu-Glu$_5$-Ala-Tyr-Gly-Try-Met-Asp-Phe-NH_2
1 2 3 4 5 6-10 11 12 13 14 15 16 17

The entire range of biological activities of the natural gastrins is also displayed by the C-terminal tetrapeptide amide (Tryp-Met-Asp-Phe-NH_2). According to Morley,[26] as many as 500 analogs of this peptide have been synthesized at Imperial Chemical Industries, and one of these, pentagastrin (Peptavlon, ICI 50,123), is now in clinical use in the United Kingdom for the assessment of gastric secretory function. While there was considerable freedom with regard to substitution of the tryptophane, methionine and phenylalanine positions, replacement of the aspartic acid side chain with the 5-tetrazoylmethyl moiety (I; Z = benzyloxycarbonyl) was the only change at that position not leading to loss of activity,[27] confirming previous observations in other systems that carboxyl and tetrazoyl groups are bio-isosteric.[28,29] The structure-activity relationships suggest that the N-terminal tridecapeptide of gastrin is responsible for transport, the tryptophane, methionine and phenylalanine amide units of the C-terminal tetrapeptide for receptor binding, and the aspartic acid residue for proton transfer. Although inhibitors of gastrin-induced acid secretion have not been discovered, it is of interest that several tetrapeptide derivatives were powerful inhibitors of gastrin-induced stomach contractions *in vitro*.[27]



I

While the primary action of gastrin is stimulation of gastric acid secretion from the oxyntic glands of the stomach, most of the effects of secretin seem to be directed toward the prevention of excessive acidification of the duodenum. Thus, secretin causes (a) stimulation of a copious flow of pancreatic juice with a high bicarbonate content and low enzyme concentration, (b) stimulation of biliary bicarbonate flow, (c) inhibition of gastrin-stimulated acid secretion and (d) inhibition of gastric motility.[30] Pancreatic volume flow and bicarbonate output have been shown to be quantitatively related to the rate of infusion of acid into the duodenum and to the amount of circulating endogenous secretin.[31] In dogs with Heidenhain pouches and denervated antral pouches, secretin decreased acid secretion in response to small doses of

histamine, methacholine, gastrin pentapeptide, and to the antral and intestinal phase of gastric secretion.[32] Secretin may also be involved in the physiological control of pepsin secretion, as evidenced by the observation that combination with histamine or gastrin pentapeptide caused a sharp rise in pepsin output.[33] Maximal bicarbonate secretion of normal subjects and duodenal ulcer patients in response to continuous intravenous infusions of secretin were not significantly different, indicating that pancreatic function is unimpaired in duodenal ulcer disease.[34]

Like secretin, CCK-PZ is isolated from the duodeno-jejunal mucosa and its physiological release is presumably controlled by the same factors that are responsible for the release of secretin. The primary effects of CCK-PZ are generally considered to be stimulation of pancreatic enzyme secretion and contraction of the gall bladder. CCK-PZ has also been reported to inhibit gastric motility,[35,36] cause contractions of the small intestine,[37,38] relax the sphincter of Oddi,[39] and stimulate pancreatic enzyme synthesis.[40] In addition, CCK-PZ displays gastrin-like effects on acid secretion in humans,[41] in dogs with denervated gastric pouch or gastric fistulae,[42] and in the isolated gastric mucosa of the bullfrog.[43] Whereas concomitant administration with methacholine caused a synergistic effect in dogs,[42] CCK-PZ depressed acid secretion evoked by exogenous gastrin, gastrin pentapeptide, histamine, and in response to the antral and intestinal phase of gastric secretion,[44-46] suggesting that it may be a physiological regulator of the hormonal phase of gastric secretion.

A glucagon-like substance has been identified in the gastrointestinal tract. It is released during enteric administration of glucose, cross-reacts with pancreatic glucagon in the immunoassay, and stimulates insulin secretion. However, the material present in extracts of canine jejunum differs from pancreatic glucagon in molecular size, lack of hyperglycemic and glycogenolytic activity, and failure to increase hepatic intracellular levels of cyclic 3',5'-AMP.[47] It has been shown that secretin and CCK-PZ, like glucagon, possess insulin releasing properties,[48-51] but it is not known if these effects have physiological significance.[51,52]

Histamine - Although it is still controversial whether or not histamine is the physiological mediator of the effects of gastrin and other stimulants of gastric secretion, further evidence favoring this hypothesis has been obtained. Confirming earlier observations by Levine,[53] the histidine decarboxylase inhibitor brocresine (NSD-1055, 4-bromo-3-hydroxybenzyloxyamine) effectively inhibited basal as well as gastrin- and gastrin pentapeptide-induced acid secretion in rats, but not secretion in response to histamine.[54] Reilly and Schayer demonstrated marked inhibition of histamine formation in the mouse gastric mucosa with MK-785 (the hydrazino analog of histidine), brocresine and p-toluenesulfonylhydrazine, a previously unreported histidine decarboxylase inhibitor.[55] Evidence was also obtained for the presence of specific histidine decarboxylase activity in the human and monkey gastric mucosa

as well as in human gastric carcinoid tissue.[56] Furthermore, gastric acid output was strongly reduced by administration of brocresine in patients with the Zollinger-Ellison syndrome, a disease characterized by gastric hypersecretion and severe peptic ulceration resulting from a gastrin secreting pancreatic tumor.[57,58]

Effects of Prostaglandins on Gastric Secretion and Gastrointestinal Motility

Prostaglandins have been identified in the gastrointestinal tract of a variety of species, and spontaneous acid secretion in the rat has been shown to be accompanied by the release of PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$.[59-61] The fact that cholinergic stimulation increases this release[62,63] and exogenous PGE_1 inhibits both basal gastric acid secretion and secretion in response to vagal stimulation[59] suggests that prostaglandin release serves to prevent excessive gastric acid output. PGE_1 also inhibited histamine- and gastrin pentapeptide-induced gastric acid secretion in anesthetized rats,[59] and reduced volume, acid and pepsin output in pylorus-ligated rats[64] as well as Pavlov and Heidenhain pouch dogs.[65,66] Furthermore, it reduced the incidence and severity of steroid and Shay ulcers in rats.[64,66] PGA_1, on the other hand, depressed food- but not histamine-induced gastric secretion in dogs.[65]

In contrast to the effects in animals, PGE_1 administered orally to human volunteers failed to decrease pentagastrin-induced gastric acid secretion at doses which affected gastrointestinal motility.[67] It is possible that it did not reach the site of action by this route of administration, or that the doses used were insufficient to inhibit secretion. Alternatively, it may be that the human stomach, which contains PGE_2 rather than PGE_1,[68] is not responsive to PGE_1 and that PGE_2 might inhibit secretion in man.[67]

The mechanism of the antisecretory action of the prostaglandins is not known. On the basis of evidence that cyclic 3',5'-AMP stimulates acid secretion in the isolated frog gastric mucosa[69,70] and perfused stomach of the anesthetized rat and potentiation of this effect by theophylline,[59] it has been suggested that the cyclic nucleotide may be the mediator of hormonal-induced gastric acid secretion. By analogy to their actions in other tissues, prostaglandins therefore could interfere with the accumulation of cyclic 3',5'-AMP in the parietal cells of the stomach.[71,72] However, in man and dog cyclic 3',5'-AMP reduced volume and output of acid in response to histamine and histalog, and had no effect on basal gastric secretion.[73] Conceivably, the prostaglandins, cyclic 3',5'-AMP and other adenine nucleotides exert their inhibitory effects on gastric secretion in these species indirectly by decreasing local gastric blood flow.[64,73] This possibility is supported by observations indicating that increased gastric blood flow in dogs is associated with the actions of secretagogues (acetycholine, histamine, gastrin) and decreased flow with the actions of inhibitors of gastric secretion (catecholamines, CCK-PZ, secretin).[74,75] Furthermore, gastric flow is

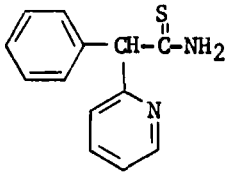
increased by vagal stimulation but decreased by sympathetic stimulation.[75]

While PGE_1 and PGE_2 cause contraction of longitudinal gastrointestinal muscle in several species, circular muscle is inhibited.[68,76,77] This and the fact that these substances are released during experimental peristalsis suggests that prostaglandins have a part in the control of gastrointestinal motor activity.[61,62]

The effects of PGE_1 and cyclic 3',5'-AMP in man differ in that the former stimulates and the latter decreases intestinal motility.[67,73] In this regard it is of interest that prostaglandins have been implicated in the diarrhea seen in subjects with amine-peptide secreting tumors.[78]

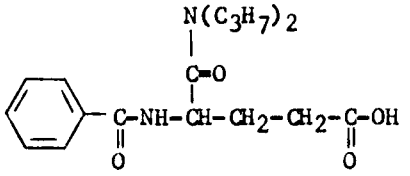
Miscellaneous Agents with Antisecretory or Antiulcer Activity

SC-15396 - The understanding of the action of gastrin in gastric secretion and the relatively ready accessibility of gastrin and gastrin peptides has stimulated the search for gastrin antagonists. Such a program has afforded SC-15396, 2-phenyl-2-(2-pyridyl)-thioacetamide, a non-anticholinergic antisecretory agent.[79,80] SC-15396 inhibited secretion induced by gastrin and pentagastrin in dogs at doses of 1 mg/kg, whereas histamine-induced secretion was not affected.[81] Inhibition of the gastrin response in anesthetized rats was surmountable by increasing doses of gastrin, suggesting competitive inhibition.[79] On the basis of this apparent selectivity for gastrin-induced secretion, SC-15396 has been termed antigastrin.[81] However, according to more recent reports, higher doses of SC-15396 also suppress histamine- and insulin-induced gastric secretion in Heidenhain pouch dogs,[80,82,83] and histamine-induced secretion in Heidenhain pouch rats.[84] Moreover, SC-15396 failed to inhibit the stimulating action of gastrin on histamine formation in the glandular part of the rat gastric mucosa, a finding that appears to be inconsistant with a specific antigastrin effect.[5,85] Kahlson et al. suggested that SC-15396 reduces the responsiveness of the parietal cells to histamine.[84] This raises the possibility that it is a histamine H_2-receptor antagonist.[86,87] Gastrin tetrapeptide-induced gastric secretion in cats was not inhibited, suggesting a species difference.[88] No human studies have been reported with this compound.



SC-15396

Xylamide - This new antiulcer agent(N-benzoyl-N,N-di-n-propyl-DL-isoglutamine) was marketed in Europe in 1967 under the trade name Milid (Rotta). Its principal effects are inhibition of gastric acid and pepsin secretion, and papaverin-like spasmolytic activity. Gastric acid secretion in response to pyloric ligation or chemical stimuli such as gastrin, histamine and insulin is inhibited in a variety of species including rat, guinea pig and dog.[89-92] Potency in the Shay rat is approximately one hundredth that of atropine.[93] Although being particularly effective



xylamide

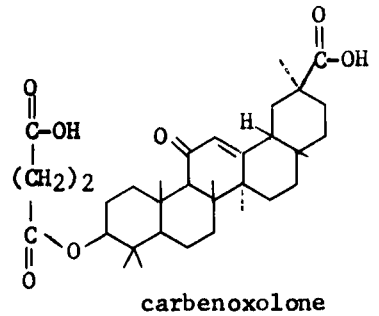
against insulin-induced gastric secretion, which is known to be vagus-mediated, xylamide appears to decrease secretion by a non-anticholinergic mechanism at sites distal to the parasympathetic nerve terminals. Activity is confined to the gastric mucosa; lacrimal, biliary, salivary and parotid gland secretions as well as duodenal motility are not affected.[93]

In man, daily administration of 800 - 1600 mg of xylamide in divided doses is reported to decrease acid secretion, alleviate pain associated with gastro-duodenal disorders and cause healing of both gastric and duodenal ulcers, without significant side effects.[94]

A series of analogs with modified or interchanged functional groups have been reported, but these generally displayed decreased anti-secretory activity or greater acute toxicity.[92,93]

It was reported some years ago that L-glutamine has ulcer healing effects in man.[95] These observations were recently confirmed in rats in which it was the most effective among a series of 18 amino acids in preventing the formation and promoting the healing of stress-produced ulcers.[96] In contrast to xylamide, however, L-glutamine neither decreases gastric acid secretion nor affects gastric motility.

Carbenoxolone Sodium - Much of the information on this drug has been summarized in the proceedings of a symposium,[97] held in late 1967, and in recent reviews.[98,99] Carbenoxolone (Biogastrone) is the water soluble disodium salt of the hemisuccinate of glycyrrhetinic acid, the diglucuronide of which, glycyrrhizic acid, occurs in licorice. The agent is known to have anti-inflammatory activity (1/3 that of hydrocortisone) in intact,



carbenoxolone

but not adrenalectomized experimental animals. In contrast to other acidic anti-inflammatory agents which cause ulceration, carbenoxolone is reported to prevent formation and accelerate healing of gastric ulcers in animals and humans. However, the incidence of side effects, primarily aldosterone-like fluid retention and electrolyte disturbances, appears to be appreciable. While thiazides can alleviate these, co-administration of the aldosterone antagonist spironolactone seems to prevent both the therapeutic action and side effects.[100]

Carbenoxolone in tablet form is rapidly absorbed from the stomach

and excreted essentially metabolized by the bile, which may explain the ineffectiveness of this preparation in duodenal ulcer. A positioned release capsule (Duogastrone), designed to release its contents in high concentration into the pyloric canal has been developed, but early clinical results are equivocal.[97,98,101,102]

Relatively little is known about the mechanism of action of carbenoxolone. It inhibits mitochondrial oxidative phosphorylation and histidine decarboxylase in vitro, but these properties are shared by the ulcerogenic anti-inflammatory agents.[103] Gastric motility and acidity, factors against which current antiulcer therapy is mainly directed, are unaffected. According to recent histological evidence, a likely mechanism of action is stimulation of mucus production.[104] Since the gastric mucosa has a greater mucus secreting capacity than the duodenal mucosa, it might be expected that carbenoxolone would be more effective against gastric than duodenal ulcers.

Licorice extract contains components with ulcer healing properties devoid of the undesirable fluid and sodium retaining effects of glycyrrhetinic acid and carbenoxolone. However, these elicit spasmolytic activity[105] and decrease gastric acid secretion,[106] and therefore may differ from carbenoxolone in mode of action.

Depepsen - It is generally appreciated that the combined action of acid and pepsin is important in the pathogenesis of peptic ulcer. The parietal cell system in the gastric mucosa, which secretes water, acid and electrolytes, and the peptic cell system, which is responsible for the synthesis and secretion of pepsinogens, are generally intimately integrated. However, in contrast to the numerous studies dealing with the secretion and inhibition of secretion of gastric acid, comparatively little attention is being directed toward the inhibition of gastric proteolytic activity.

Cook and Drill have described in detail the pharmacology of depepsen (SN-263), a synthetic sulfated polysaccharide which is essentially devoid of the anticoagulant activity generally associated with this class of compounds.[107] Depepsen inhibits pepsin proteolytic activity and prevents ulceration in a variety of animal models, but does not affect acid output and gastric motility. Although not as effective as anticholinergics, efficacy has been demonstrated in duodenal ulcer patients at oral doses of 200 - 800 mg.[108] Addition of anticholinergic drugs increases efficacy, and such combinations have been reported to prevent the recurrence of duodenal ulcers. It has been suggested that depepsen may be most useful in patients in whom anticholinergic drugs are contraindicated.[109]

Gastrone - It is known that gastric juice and saliva of a variety of species contain gastrone, a substance that depresses hydrochloric acid secretion in rats and dogs.[110,111] Recent studies indicate that gastrone of human anacid gastric juice is composed of at least two materials that differ chemically, electrophoretically, immunologically, and in inhibitory

potency.[112] One of these, having a molecular weight greater than 100,000, resembles mucosubstances in electrophoretic mobility, carbohydrate content and resistance to peptic digestion. Intravenous administration of doses of 250 μg to pylorus-ligated rats caused 50% inhibition of gastric acid output. The other component appears to be an undialyzable glycoprotein with a molecular weight between 10,000 and 40,000. This is susceptible to peptic digestion, has properties of a γ-globulin, and is more potent than the mucosubstance-related material in that gastric acid secretion in the pylorus-ligated rat was inhibited at doses as low as 7 to 48 μg. The physiological significance and the mechanism of action of gastrone are unknown.

Other Agents - Bass and Patterson studied the gastric secretory (volume) responses of drugs affecting adrenergic mechanisms in pylorus-ligated rats.[113] Norepinephrine, epinephrine and isoproterenol were found to be potent antisecretory agents. α-Adrenergic blocking agents also exerted potent antisecretory effects, and the potencies of these drugs paralleled their catecholamine releasing properties. Agents that inhibit the uptake of norepinephrine into adrenergic nerve terminals were generally more potent than those that interfere with its release.

Geranyl farnesylacetate (gefarnate, gefarnil), a member of a series of farnesylacetates, studied in detail by Adami and co-workers several years ago,[114] has been shown to possess preventive and curative effects on stress-produced ulcers.[96] Similarly, vitamin A has been reported to possess antiulcerogenic properties in rats.[115]

In follow-up of the observation that 2',2'-bipyridine exhibited potent antisecretory activity in rats and dogs, Bass et al. examined a variety of chelators to determine if there is a correlation between chelating properties and antisecretory activity. Evidence for such a correlation was not obtained.[116]

Aspirin-Induced Gastric Ulceration

Considerable attention has been directed toward the problem of salicylate-induced gastric hemorrhage and ulceration. Bleeding from the gastrointestinal tract occurs in the majority of people taking repeated therapeutic doses of aspirin.[117] Davenport has made the interesting suggestion that acetylsalicylic acid injures the lipid-protein layer of the gastric mucosa as it is absorbed.[118] The resulting increased mucosal permeability permits hydrogen ions to diffuse from the lumen into the mucosa and cause damage to capillaries rendered sensitive through the liberation of histamine. This hypothesis is supported by the observation that gastric irrigation with aspirin in Heidenhain pouch dogs caused back-diffusion of hydrogen ions and release of fluid, sodium and potassium ions into the lumen. In addition, administration of antacids and anticholinergic drugs decreased aspirin-induced mucosal damage.[119] It has also been suggested that aspirin increases epithelial cell exfoliation,[120] reduces the secretion of mucus[121] and alters its composition[122] and physicochemical properties.[123]

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Chapter 7. Pulmonary Drugs

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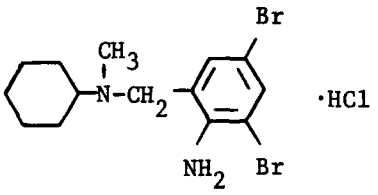
Pulmonary Surfactant - Studies conducted largely during the last two decades have established the presence of a cellular lining film on the inner aspect of the alveolar surface. A surfactant component of this film is believed to function physiologically to lower surface tension of the alveolar wall, especially when the surface area is reduced, thereby promoting alveolar stability.[1] The pulmonary surfactant is thought to be synthesized, and perhaps also stored[2] and cleared from the lung,[3] by the epithelial cells of the alveoli[2,4] or by the cells lining the terminal bronchioles.[3] Some studies indicate the chemical nature of the surfactant to be that of a lipoprotein.[5] However, some recent investigators[6] have concluded that protein is not an essential component. An insufficient amount or derangement of function of pulmonary surfactant is implicated in the respiratory distress syndrome of the newborn, although both the pathogenesis[7] and therapy[8] of this condition remain matters of controversy. Current concepts concerning the respiratory distress syndrome, and the role of pulmonary surfactant therein, have been recently reviewed.[9]

Mucolytics - The problems presented by thick, tenacious sputum are central to the treatment of chronic bronchitis. There is an important need for effective mucolytics, particularly orally effective compounds.[10] The mucolytic-expectorant bromhexine[11] (I), N-cyclohexyl-N-methyl-(2-amino-3,5-dibromobenzyl)ammonium chloride, was shown earlier to fragment the acid mucopolysaccharide fibers in sputum, thereby decreasing sputum viscosity. These findings have been corroborated by the study of Bruce & Kumar[12] who examined oral effectiveness (8 mg t.i.d.) over a ten day period in 5 normal subjects with uninfected sputum. Marked fragmentation of the acid mucopolysaccharide fibers and decreased sputum viscosity occurred by the end of the treatment period, followed five days later by return to control levels or above.

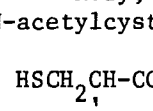


I

Shimamoto and Honjo[13] concluded from animal studies that bromhexine affects tracheobronchial secretions by an action on central nervous system structures, probably in the areas of the emetic center in the medulla oblongata occurring both as a direct effect and indirectly via stimulation of the gastric mucous membrane. Other animal tests indicated bronchial secretions were increased by bromhexine as a result of an improved permeability ratio between the bronchial mucous membrane and its blood supply.[14] The lytic effect of bromhexine on human bronchial mucosa, observed in vitro by electron microscopy, appears due to increased secretion

of lysosome-like granules by the serous epithelial cells of the bronchial glands.[15] These granules adhere to the bronchial goblet cells and lyse their mucous secretion. Bürgi, et al.[16] determined that bromhexine markedly increased the γ-globulin fraction of the soluble proteins from human bronchitic sputum. In view of the postulated relationship between the concentration of γ-globulin in the sputum and the humoral defense mechanism at the surface of the bronchial mucosa,[17] the authors speculate that the increased γ-globulin induced by bromhexine could explain, at least in part, the beneficial effects of the compound in patients who produce no sputum.

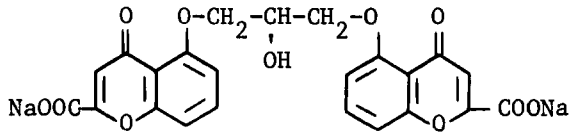
Kory, et al.[18] reported that the pronounced mucolytic action of N-acetylcysteine (II) in patients with chronic bronchitis was not lessened by concomitant nebulization with racemic epinephrine. However, the bronchospasm previously observed with topical administration of N-acetylcysteine alone did not occur. For similar reasons, an aerosol combination with a bronchodilator has also been recommended for pancreatic dornase.[19]



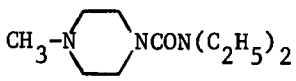
II

An in vitro study, employing a cone-plate viscometer for the evaluation of viscosity,[20] confirmed the effective lytic action of sulfhydryl compounds on both purulent and mucoid sputa whereas dornase was effective only on purulent sputum.[21]

Immunological Aspects - In the past year, the immunologic approach to asthma therapy has centered around clinical studies employing disodium cromoglycate (III), a chromone derivative whose pharmacology was initially



III



IV

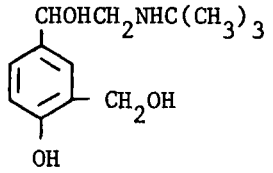
published the year before.[22] The compound is inhaled from a capsule containing 20 mg disodium cromoglycate as a micronized powder with an equal amount of lactose as a diluent, with[23,25,27-30] or without[24,31] 0.1 mg isoproterenol to prevent the potential bronchospastic action of the dry powder. The usual dose is 4 capsules daily, delivered by suction (inspiratory motion) through a special plastic inhaler. In several double-blind studies employing placebo controls, this dosage regimen produces significant subjective improvement and reductions in the corticosteroid and bronchodilator requirements of allergic asthmatics.[23,25,26,30,31] Improvement in ventilatory function, as measured by spirometry, was less prominent or absent. This latter finding leads to reservations about labeling disodium cromoglycate as a major advance in asthmatic therapy.[28,32] The potential danger of a severe relapse upon cessation of cromoglycate therapy in those asthmatics allowed to withdraw from steroid treatment has also been stressed.[26,28]

Aside from its potential in the armamentarium of asthma therapy, disodium cromoglycate represents a valuable tool for studying the immunological mechanisms involved in respiratory allergic disease.[24,33] Pepys and his colleagues[24] found the prophylactic administration of disodium cromoglycate in humans inhibited both the immediate reagin-mediated type-I allergic response to inhaled specific allergen and the later-developing type-III asthmatic and febrile reactions characterized as precipitin-mediated. Thus, a relationship between type-I and type-III reactions, with the former influencing the development of the latter, is implied.[34]

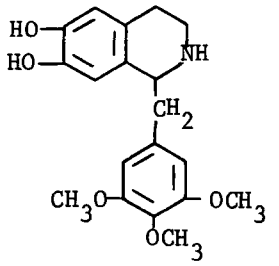
Orange and co-workers evaluated the effects of disodium cromoglycate and diethylcarbamazine (IV) on the immunologic release of chemical mediators in rats passively sensitized by homologous antiserum. Diethylcarbamazine was reported in an earlier, uncontrolled study[35] to elicit marked improvement in fourteen of fifteen intractable asthmatics. In the immunologic tests, disodium cromoglycate inhibited the antigen-mediated release of histamine but not the immunologic release of slow reacting substance of anaphylaxis (SRS-A).[36] In contrast, diethylcarbamazine inhibited the release of SRS-A[37,38] but did not block the elaboration of histamine.[38] Neither agent prevented the action of the chemical mediators on the end organ[22] nor interfered with the antigen-antibody interaction itself.[36-38] Rather each agent inhibited selectively, at some subsequent step in the reaction process, the formation or release of a chemical mediator. The recommendation of additional clinical trials with these agents, singly and in combination, seems justified.[39]

Sympathomimetic-Bronchodilators - Since bronchiolar smooth muscle is innervated by the adrenergic system, sympathomimetic amines can generally be used with success as relaxants regardless of the nature of the spasmogen. The sympathomimetics elicit their effect by β-adrenergic stimulation; hence the use of such agents is commonly attended with other β-adrenergic stimulant effects, such as cardiac acceleration and tremors. Although it is commonly assumed that inhaled sympathomimetics act directly on bronchiolar smooth muscle, it is possible that their effect is also "systemic", arising by adsorption and redistribution.

Structural analogues of epinephrine and isoproterenol continue to be synthesized and evaluated in the search for agents with fewer systemic side effects and better duration of action than the prototype catecholamines. Salbutamol (V) is representative of a new group of β-adrenergic



V

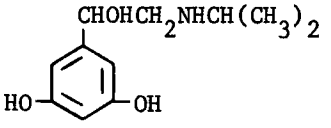


VI

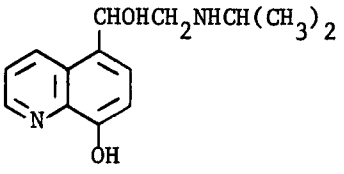
stimulants, formally derived from saligenin.[40] Two series of compounds are possible, one wherein the ethanolamine side chain is meta to the hydroxymethyl group and the second where the side chain is para. Only compounds of the first series evidence significant adrenergic actions. This is comparable to findings with the two series of "mixed catechols" which contain the alkanesulfonamido group and a phenolic hydroxyl group.[41] Salbutamol is not a substrate for COMT. Its duration and intensity of bronchodilator action are reported to excede those of isoproterenol. It is also suggested that salbutamol exhibits a greater specificity for bronchiolar smooth muscle than isoproterenol.

A tetrahydroisoquinoline derivative, trimethoquinol, 1-(3,4,5-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroquinoline (VI), has recently been reported to be an extremely effective sympathomimetic bronchodilator.[42] An arylmethyl group in the 1-position of the tetrahydroisoquinoline ring is necessary for maximum activity in this structural family, as are the hydroxyl groups in the 6- and 7-positions. Unsaturation at the 1,2-position and methyl groups in the 2- and 3-positions of the tetrahydroisoquinoline system eliminate significant direct-acting β-adrenergic stimulant activity. The authors suggest that the β-adrenergic activity of trimethoquinol and related structures cannot be explained in light of some existing theories for depicting activity at β-adrenergic receptors.

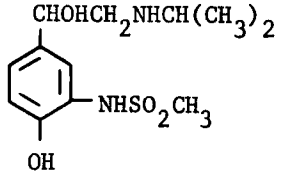
New studies have been reported with other sympathomimetic bronchodilators, such as metaproterenol (VII),[43] quinterenol (VIII),[44] and soterenol (IX).[45]



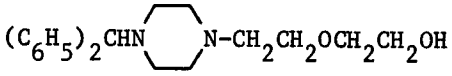
VII



VIII



IX

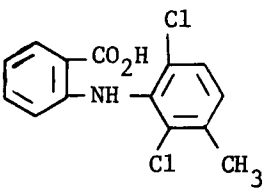


X

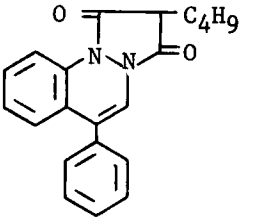
Miscellaneous Bronchodilators - Chlordiazepoxide has been reported to inhibit bronchospasms induced by serotonin, histamine, acetylcholine, bradykinin and albumin. The action is partly due to a direct relaxant effect and partly to an adrenergic β-receptor stimulating action. This pronounced action on bronchiolar smooth muscle, especially against histamine, is in contrast to the weak effects of this drug on other tissue beds.[46]

Decloxizine (UCB-1402) (X), in addition to the antihistaminic and anti-serotonin actions expected as a result of its structure, has been reported to possess a direct-acting muscolotropic bronchodilator action equivalent to that of isoproterenol.[47]

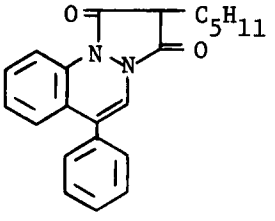
Among a series of non-steroidal anti-inflammatory agents evaluated intravenously for inhibition of bradykinin- and anaphylaxis-induced bronchoconstriction,[48] the most potent compounds were meclofenamate (XI), Scha 87/2 (XII) and cinnopentazone (Scha 306) (XIII).[49]



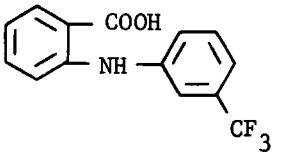
XI



XII



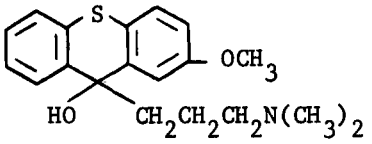
XIII



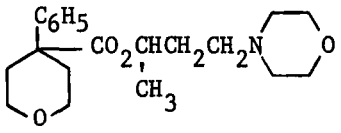
XIV

In another study, the "fenamates" were shown to antagonize the response of human isolated bronchial muscle to SRS-A and PGF_{2a}, particularly the latter.[50] Meclofenamate (XI) was more active than flufenamic acid (XIV) and both were more active than phenylbutazone or aspirin. Evaluation of flufenamic acid in partially refractory asthmatics failed to reveal any significant beneficial therapeutic effect.[51]

Anti-Tussives - A thioxanthene, meprotixol (XV), was evaluated clinically

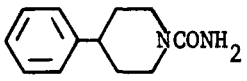


XV

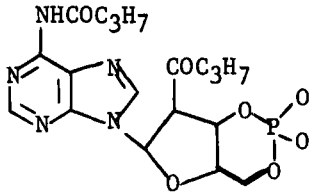


XVI

and found to be as effective as codeine, without the concomitant respiratory depression.[52] A basic ester, phenhydropyxylate (XVI), was reported to be more effective than dextromethorphan.[53] Laboratory studies, including some involving conscious animals, have shown AH-1932[54] to be as active as codeine. This arylpiperidine derivative (XVII) is presumed to act centrally. It has no effect on respiration or gastrointestinal motility.



XVII



XVIII

Beta-Blocking Agents - There is now considerable laboratory and clinical data showing that β-adrenergic blocking agents can have a bronchoconstrictive and bronchospastic action. This is hardly unexpected, since these agents can interfere with the relaxant effect of endogenous amines and/or the action of exogenous β-adrenergic stimulants.

A study of the action of dibutyryl cyclic 3',5'-AMP[55] (XVIII) on a tracheal chain preparation showed that this derivative of cyclic 3',5'-AMP was a potent relaxant.[56] Dibutyryl 3',5'-AMP was more active than isoproterenol and its action was not blocked by the β-adrenergic blocking agent, propanolol. In contrast to cyclic 3',5'-AMP, the dibutyryl derivative was not a substrate for phosphodiesterase. Cyclic 3',5'-AMP itself exhibited no relaxant action on the tracheal preparation, presumably as the result of its inability to penetrate cellular structures.

Steroids - The usefulness of corticosteroids in allergic respiratory states remain limited by the varied and sometimes serious side effects associated with this therapy, as well as by rebound phenomena at withdrawal.[57] Maunsell et al. found no evidence of tolerance to the therapeutic effect of long-term oral corticosteroid treatment in 170 asthmatic patients followed for periods up to 13 years.[58] There was, however, a 20% incidence of dosage-related side effects in this study and exacerbation of asthmatic symptoms subsequent to drug withdrawal in 11 of

52 cases. Nash and Jacomb[59] concluded from a comparative study in 21 asthmatic patients that repository intramuscular corticosteroid therapy can effectively replace oral administration and permit decreased total steroid dosage and increased periods of remission. Another clinical study in allergic asthmatics indicated sublingual betamethasone afforded a more rapid onset and greater duration of effect than oral drug, as measured by the change in numbers of circulating eosinophils.[60] Chervinsky[61] compared the onset and duration of action of four steroid preparations, including a rapidly absorbed steroid phosphate, a less rapidly absorbed acetate form and a phosphate-acetate mixture, in a series of 100 allergic rhinitis patients. The combination product afforded no advantage over the long-acting steroid acetate alone.

A synthetic adrenocortical stimulant, depot-tetracosactrin, elicited no allergic reactions when administered over a period of 8 months to 9 asthmatic patients with known severe hypersensitivity to natural corticotropin.[62] The synthetic polypeptide produced sustained increases in plasma and urinary levels of 17-hydroxycorticosteroids and had a significantly longer duration of action than natural corticotropin.[63]

Additional evidence for a corticosteroid effect in an allergic respiratory disease was furnished by a study[64] in 52 asthmatic patients, showing an inverse relationship between severity of asthma and steroid reserves in the adrenal cortex. In view of the prominent anti-inflammatory action of the corticosteroids, it has been proposed that the steroids act only on the inflammatory components of bronchial obstruction.[65] However, recent animal studies indicate other mechanisms may be involved in the bronchodilator effects of corticosteroids. Carrillo and Aviado[66] determined the effects of hydrocortisone and dexamethasone on the histamine content and the mechanical properties of the lungs, in both sensitized and non-sensitized rabbits, and concluded that the steroids induce broncho-dilation both by depletion of lung histamine and by direct bronchiolar smooth muscle relaxant action.

Ito and Aviado[67] reported the effects of progesterone administration on pulmonary emphysema induced in rats by a combination of tracheal constriction and intra-tracheal injection of phytohemagglutinin. Progesterone pretreatment for a period of 7 weeks prevented the increase in functional residual capacity and the development of the histological picture of emphysema (i.e., air space distention and thickening of alveolar wall) which occurred in placebo-treated rats. The increase induced in pulmonary resistance was not prevented by progesterone, suggesting that the increase in airway resistance was a causative factor in, rather than the result of, the alveolar lesions produced in this experimental model. Progesterone also elicited no change in pulmonary resistance in a series of 8 obese hypoventilation patients, although it was efficacious therapy in this syndrome because it increased total alveolar ventilation.[68]

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Chapter 8. Vasodilator and Vasoconstrictor Agents

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Vascular smooth muscle tone or degree of vasoconstriction, is a critical determinant of pressure within blood vessels and the flow of blood through tissues. Resistance to blood flow is offered primarily by small arteries and arterioles - the precapillary resistance vessels. Blood flows through these vessels to the capillaries, where exchange of fluid and solutes (the "nutritive" function) occurs, and subsequently to the postcapillary resistance vessels that form part of the venous system. Constriction of precapillary vessels decreases intra-capillary pressure and promotes movement of fluid into the vessels from surrounding tissue spaces; postcapillary constriction has the opposite effect[1]. The contribution of postcapillary vessels to total flow resistance, although small, is of considerable significance, for transcapillary fluid exchange is determined to an important degree by the ratio of precapillary to postcapillary resistance[1]. Whether induced by a drug or by disease it is thus apparent that altered tone in the various segments of the vascular tree can markedly influence not only the supply of blood to a given organ or vascular bed but also tissue nutrition.

Many drugs with diverse pharmacological actions ultimately alter vascular tone. Among those that reduce vascular tone can be listed antihypertensive agents as well as drugs used to treat peripheral vascular diseases. Agents causing vasoconstriction find clinical application primarily in conditions associated with unacceptably low blood pressure or tissue perfusion (e.g., hemorrhagic or cardiogenic shock). Since antihypertensive drugs are described in an accompanying chapter of this volume and antianginal agents were reviewed in three previous volumes, we will confine our attention to peripheral vasodilator and vasoconstrictor agents that do not fall clearly into the general classes of drugs referred to above.

Vasodilator and vasoconstrictor agents may act directly on the basic contractile mechanism of vascular smooth muscle or indirectly by affecting one or more of the pharmacological receptors that ultimately influence vascular tone; several drugs have both direct and indirect effects. Under physiological conditions, vascular tone is maintained by tonic activity in sympathetic nerves. Drugs decreasing such tone by, ganglionic or adrenergic blockade, are candidate vasodilator agents. It is therefore apparent that considerable overlap exists among antihypertensive drugs and those whose effects

are directed more specifically toward the treatment of peripheral vascular disease.

Drugs with Predominantly Direct Action - Octapressin, a synthetic analog of lysine vasopressin, has been studied in conditions of hypotension in man[2]. In low doses, the peptide caused renal vasodilatation while higher doses resulted in preferential extrarenal vasoconstriction with consequent redistribution of blood flow to the kidney. Accordingly, the drug may have application in conditions associated with hypotension and renal vasoconstriction (a combination that very often coexists in conditions of shock[2]). Octapressin has been used in the past clinically and experimentally to treat circulatory shock[3] and is particularly effective in microcirculatory failure[4].

The nona-peptide bradykinin, a potent arteriolar smooth muscle dilator, has continued to be the subject of numerous experimental observations and a review of structure activity relationships among bradykinin analogs has appeared[5]. In recent years, there has been increased interest in the action of bradykinin on venous tone. The action of bradykinin on venous segments *in vitro* is dependent on the site from which the venous vessel was removed. Thus, while bradykinin relaxes arteriolar smooth muscle it was shown to contract venous strips of dog femoral and mesenteric blood vessels[6]. This venoconstriction was uninfluenced by α- or β-adrenergic blocking agents and was therefore classified as a direct action[6]. Confirmation of a direct venoconstrictor effect was offered by Bobbin and Smith[7] who noted that adrenergic blocking or antihistaminic drugs failed to reduce bradykinin-induced constriction of rabbit ear vein. In addition, this study[7] eliminated the possible mediation of 5-hydroxytryptamine or acetylcholine in the venoconstrictive action. A detailed study[8] in which it was reported that peripheral vasodilatation and lowered aortic pressure and systemic vascular resistance caused by bradykinin in the dog was unaffected by autonomic blockade, supports the concept that the entire circulatory response to the peptide is produced by direct effects on vascular smooth muscle. An unanswered but intriguing question is why bradykinin should exert qualitatively different effects on consecutive vascular smooth muscle segments.

A series of bradykinin analogs containing the simple secondary amino acid, sarcosine, in place of the cyclic secondary structure, proline, was prepared[9] to determine whether modification of the biologic activity of bradykinin might be related to steric factors. 2-Sarcosine bradykinin was the most active analog in increasing hind limb blood flow in the dog, although its activity was at most only about 30% that of synthetic bradykinin. The 3-sarcosine derivative of bradykinin

had only 5-10 per cent of the activity of the penta peptide on blood flow but was equiactive in lowering the blood pressure of the guinea pig. This degree of specificity is intriguing since it suggests the possibility that other structural modifications of the bradykinin molecule may yield compounds that are active specifically on skeletal muscle vascular beds.

Kallidin, the lysine-homologue of bradykinin, was as effective as bradykinin in increasing digital volume in man[10]; both peptides may act by closure of arterial venous anastomosis which results in shunting of blood into capillary beds. In the same study, the peptide eledoisin was twice as effective as either bradykinin or kallidin in increasing total digital volume[10]. Replacement of the N-terminal lysine of kallidin by neutral or basic amino acids caused a slight decrease in vasodilatory activity[11]. Replacement of the N-terminal lysine by an acidic amino acid, l-glutamic acid, did not influence significantly the biological activity of the kallidin molecule as measured either on isolated guinea pig ileum or on guinea pig blood pressure[12]. In an attempt to increase the duration of the hypotensive action of eledoisin, the valeryl and caproyl derivatives of eledoisin were synthesized[13]. Both were less active than the parent peptide but did have a longer duration of action in dogs.

Bradykinin not only dilates arterioles but is thought to be linked with another accompaniment of the early inflammatory response, namely increased vascular permeability[14]. The effect of several bradykinin analogs on vascular permeability was studied in guinea pigs[15]. Lysine bradykinin (kallidin) was more effective in increasing vascular permeability than was bradykinin itself. Glycyl bradykinin had about the same degree of activity as the parent compound, while all others were less active.

The effects of substance P on general and coronary hemodynamics were studied using intact anesthetized dogs[16]. There was transient dilatation of systemic, pulmonary and coronary vascular beds, all of which were considered to follow the direct relaxant effect of substance P on vascular smooth muscle[16].

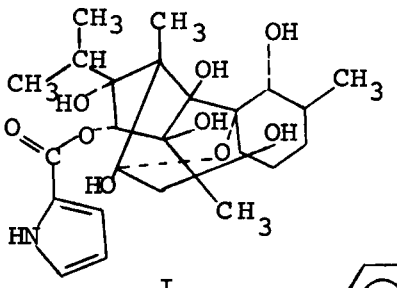
Physalaemin is a peptide found in methanol extracts of the skin of the South American amphibian Physalaemus fuscumaculatus and was first reported by Erspamer and his colleagues in 1962[17]. A recent study[18] showed that physalaemin was only 2 to 5 times more active in lowering systemic blood pressure, but was 2000 to 5000 times more active in causing dilatation of the dog femoral vascular bed than was either eledoisin, to which it is closely related structurally, or nitroglycerin. Since the vasodilatation was uninfluenced by acute or chronic

denervation of the leg the authors suggest that physalaemin has a direct action on vascular smooth muscle and propose[18] that the highly selective vasodilatation caused by this peptide merits clinical study of the drug in conditions of peripheral vascular disease in man.

In doses as low as 5 μg/kg, ryanodine (I), whose structure was recently elucidated[19], significantly increased vascular resistance in cat hind limb, spleen and tissues of the head and neck[20]. The vasoconstrictor action was selective, however, in that much larger doses were required to increase resistance in the kidney, while that of the superior mesenteric vascular bed was unchanged or decreased[20]. The vasoconstrictor action of ryanodine may be mediated centrally[20].

The prostaglandins have pronounced actions on the cardiovascular system and an extensive review of their pharmacology was published in 1968[21]. Prostaglandins of the PGE and PGA series are peripheral dilator agents while the PGF group comprises pressor and venoconstrictor agents[21]. Ducharme *et al.* reported[22] that prostaglandin $F_{2\alpha}$ increased peripheral resistance in perfused dog limbs primarily by acting on the venous side of this vascular bed. Since the pressor activity of prostaglandin $F_{2\alpha}$ in both dogs and rats was unaffected by either ganglionic blockade or by depletion of endogenous catecholamines following reserpine treatment, its mechanism involved neither sympathetic nerve activity nor release of catecholamine from adrenergic nerve endings[22]. Prostaglandin E_1 was a potent vasodilator in both guinea pig and human skin following intradermal injection, although it was not absorbed through intact skin[23]. Particularly significant was the finding[23] that prostaglandin E_1 caused vasodilatation in the skin of patients with atopic dermatitis. This is the first agent reported to cause vasodilatation in this condition. It is likely that more clinical testing will occur in the future. Also, antagonists of prostaglandins, theoretically, offer interesting possibilities for the design of both vasodilator and vasoconstrictor drugs.

Xanthinol nicotinate (II), which combines representatives of two classes of vasodilators, namely xanthines and nicotinic acid analogs, has been used to improve circulation in a number of clinical and experimental studies. Placental insufficiency, resulting from low placental circulation, was significantly improved in 13 women who received this drug[24]. Xanthinol also improved considerably lower limb circulation in 39 of 41 patients suffering from peripheral vascular disease and who had previously had lumbar sympathectomy[25]. This work[25] implies that surgical interruption of the lumbar sympathetic pathways did not totally remove vasoconstrictor tone and that xanthinol was able to decrease or abolish the remaining vasoconstriction.



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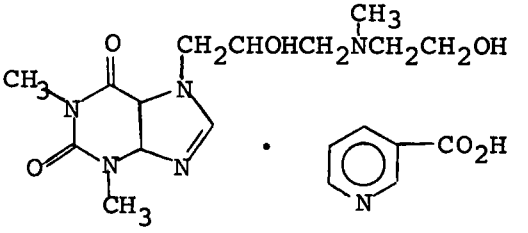

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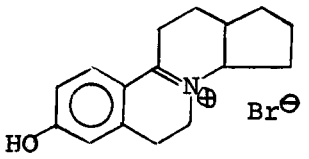

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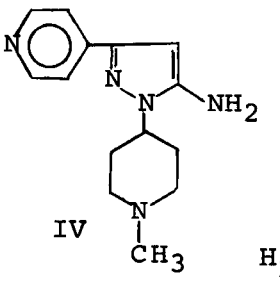

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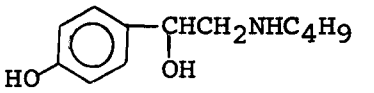

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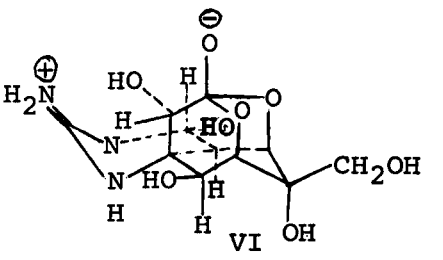

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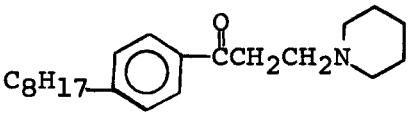

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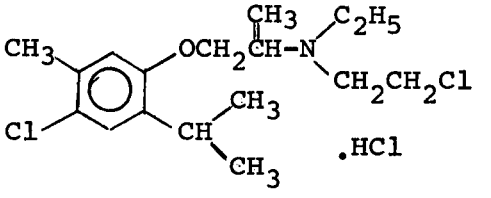

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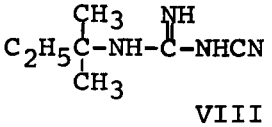

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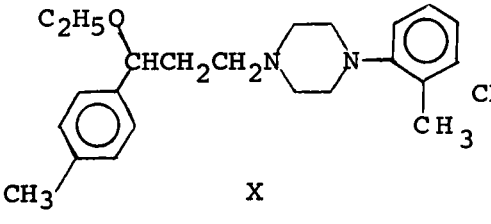

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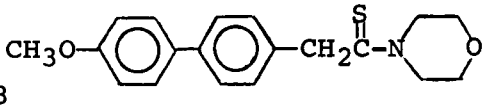

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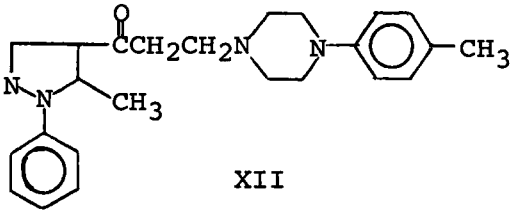

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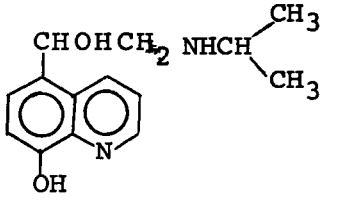

XIII

In this case, improvement in blood flow was monitored by measurement of surface temperature in the affected foot. Intestinal infarction, produced by selective ligation of the mesentery vessels in rabbits, was relieved to some extent by the drug[26].

Quindonium bromide, W 3366A (III), reported to protect dogs against hemorrhagic shock[27], was found similarly to protect rats subjected to traumatic shock[28]. Quindonium, thought to dilate blood vessels by a direct action, was effective if given before, but not after the application of the trauma (placement of the rat in a revolving drum). Another vasodilator drug thought to have a direct action on vascular smooth muscle is Ciba 31531-Ba (IV) which had a pronounced pulmonary vasodilator action in cats[29].

Bamethane sulfate (V), a dilator drug known for many years, was recently employed in two studies involving patients with obliterative atherosclerosis and intermittent claudication[30,31]. There was distinct clinical improvement in 7 patients. One patient, who failed to show improvement in lower limb circulation, nevertheless had some relief from the manifestations of concomitant cerebral arterial disease[30].

Lipsius *et al.*[32] re-evaluated the mechanism of tetrodotoxin-induced hypotension and reported convincing evidence that the drug dilated skeletal muscle vasculature by a direct action, which was unaltered by either α-or β-blocking agents or by the antihistamine diphenhydramine. The chemistry[33] and pharmacology[34] of tetrodotoxin (VI) have been reviewed.

In clinical tests involving 122 patients with various forms of occlusive peripheral arterial disease, an extract of Ginkgo biloba (Tebonin) significantly improved peripheral blood flow[35], as judged by the increased ability of these patients to walk test distances and also by direct measurement of skin and muscle temperatures, indicative of skin and muscle blood flow, respectively[35]. The same extract was also found[36] to lower arterial pressure and to dilate peripheral blood vessels including capillaries in patients suffering from the postthrombotic syndrome. Ginkgo biloba extract contains a variety of substances including acetylated flavenone- and flavenolone-glycosides; the component(s) responsible for the pharmacological activity is presently unclear.

A β-aminoketone (N-1113)(VII) was recently reported[37] to have roughly 3 times the activity of papaverine, on a weight basis, in increasing femoral blood flow in the cat. The authors suggest that since the dose reponse curves for the two drugs are parallel, they may have similar modes of action. This seems rather weak evidence on which to base a statement

concerning the mode of action of a new drug.

A close correlation was noted[38] between the ability of several lidocaine analogs to dilate perfused blood vessels of frog, dog and rat and their ability to lower carotid arterial blood pressure. Accordingly, the authors suggest that a direct effect on peripheral vascular muscle be considered as a major cause of the hypotension produced. However, they correctly point out that such an action might be expected also on the grounds that these compounds are local anesthetics. In addition, ganglionic blockade, also caused by the drugs, is likely to have contributed to the over-all hypotensive action.

Angiotensin II Antagonists - Angiotensin is one of the most potent naturally occurring vasoconstrictor agents known and is used clinically for this purpose as angiotensin II amide 5-valine. Its pronounced physiological actions have led to considerable speculation that the peptide may be involved in blood pressure regulation and that its overproduction could be linked to certain forms of hypertension. Recent reviews of this topic are available[42,43]. Accordingly, efforts to design antagonists of this peptide seem appropriate on theoretical grounds. Lidoflazine is an angiotensin II antagonist[39] which is a peripheral vasodilator in dogs[40], but finds its main therapeutic application as a long-acting coronary vasodilator[41]. Among the conditions with which angiotensin has been linked is the toxemia of human pregnancy. In full term human placentas, perfused *in vitro*, lidoflazine was the most active angiotensin II antagonist among a group of compounds tested[40] that included α blocking agents, papaverine and organic nitrites and nitrates.

Guancydine (VIII) antagonized the pressor responses to injected angiotensin and renin in the conscious, normotensive rat[44]. In contrast, responses to catecholamine were less antagonized, while those to directly acting vasoconstrictors (barium chloride and vasopressin) were unaffected. Such relative specificity of antagonism is potentially of considerable significance. Guancydine, following intraperitoneal injection, decreased renal, mesenteric and cranial resistance in anesthetized dogs[44].

α-adrenergic Blocking Agents - Thymoxamine is a selective α-adrenergic blocking agent that antagonizes norepinephrine competitively on vascular tissue. Structurally this agent is a thymoxyalkylamine similar to the benzodioxane α blocking agent, piperoxan. Specific α-adrenergic blocking activity was demonstrated in man using the "peripheral model" of pupilary dilatation in response to sympathomimetic amines[45].

The adrenergic blocking activity of 85 thymol ethers was

compared with that of thymoxamine (WV 365)[46]. The compound WV 0062 (IX), a haloalkylamine with a branched side chain, was superior as an adrenergic blocking agent to all others including phenoxybenzamine, both after parenteral or oral administration. WV 0062 was well absorbed from the intestine and was approximately one half as toxic as thymoxamine but had 15 times the therapeutic ratio of the latter. On clinical testing the drug lowered both systolic and diastolic blood pressures in hypertensive patients and normal subjects[47]. Such a compound may be of value as a peripheral vasodilator if it has specificity of action within the various vascular beds.

The ability of the α blocking agent Su 12080 (X) to increase femoral blood flow in anesthetized cats was compared[48] with that of phentolamine. The drugs were approximately equiactive in this regard and also in reversing peripheral vascular responses both to epinephrine and to norepinephrine.

Of a series of 37 1,4-disubstituted piperazine derivatives recently synthesized[49], roughly half caused vasodilatation in the femoral vascular bed of rabbit. Seven of the compounds increased femoral blood flow by over 100% as compared with control values. The most active compound of the series was a benzhydryl-piperazine. Some specificity of action with regard to the vascular bed affected was indicated by their lack of action on coronary, cerebral or renal circulation in the rabbit[49].

Sympathomimetic Drugs - The effects of a series of 2-(4-biphenylyl)ethylamines on the cardiovascular and central nervous systems were examined[50]. At doses well below toxic levels one compound (XI) caused peripheral vasodilatation in rats.

Among a group of 5,6-dihydro-7H,12H-dibenzazocines, the N-propyl and N-chloroethyl derivatives were more effective as peripheral vasodilators, in the rat hind quarters, than was azapetin, the dibenzazapine analog used as a reference standard. These compounds, representing a new tricyclic system, may offer an interesting pointer to new vasodilator drugs[51].

The Ciba compound, 1002-Go (XII), was studied[52] as a hypotensive agent. The drug caused marked peripheral vasodilatation in rats perhaps by stimulation of β-receptors since the effect was blocked by propranalol[53]. The vasodilatation may also involve sensitization of these receptors to epinephrine[52]. The compound also interfered with norepinephrine uptake by the rat heart and significantly depleted catecholamines from the heart and brain of rats, implying significant actions on the adrenergic nervous system.

Quinterenol (XIII) was found[54] to have β-adrenergic stimulant properties. Thus the drug increased hind limb blood flow and lowered total peripheral resistance in the dog to the same extent as did papaverine but had a considerably longer duration of action. All cardiovascular effects of quinterenol were blocked by the β-receptor blocking agent, propranalol.

Catecholamine analogs that were isomers of substituted phenethanolamines were tested as potential sympathomimetic amines[55]. Those members of the series in which the alkanesulfonamido substituent is meta and the hydroxy group is para to the ethanolamine side chain had pronounced actions as α- and β-adrenergic agonists. However, when these groups were transposed with reference to the ethanolamine side chain, adrenergic activity was greatly reduced[55]. The authors suggest that differences in activity depend on the necessity for an absolute spatially oriented interdependence between the catechol and amine portions of the molecule and the receptor structure itself. A number of these compounds were more effective β-receptor activators than isoproterenol, while others were more active α-receptor stimulants than norepinephrine. As the authors point out, these interesting data in themselves do not permit prediction of potential potency as vasoconstrictor or vasodilator agents since pressor activity was the sole determinant of over-all cardiovascular action used in this study; blood pressure is, of course, the resultant of effects in cutaneous, splanchnic, skeletal muscle and other vascular beds and is also greatly influenced by effects of drugs on cardiac function[55].

In summary, it is apparent that much of the research that ultimately produced vasodilator agents was in fact designed to uncover antihypertensive drugs. A major requirement of clinically useful vasodilators is a selective ability to increase blood flow in specific vascular beds. This specificity is only infrequently noted among vasodilator agents. Goldberg *et al.*[56] have described structural requirements for dopamine-like compounds that produce renal vasodilatation. Among these compounds only the N-methyl derivative (epinine) had unequivocal dopamine-like activity. Goldberg *et al.* conclude that the molecular structure required for such activity is more specific than that needed for α- and β-adrenergic effects. If such specificity of action could be attained in skeletal muscle vascular beds, the resulting drugs would almost certainly be of considerable interest as vasodilator agents for the treatment of peripheral vascular disease.

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Section III - Chemotherapeutic Agents

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Chapter 9. Antibiotics and Related Compounds

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General. Selected scientific contributions of Selman A. Waksman were published[1] to honor the nestor on his eightieth birthday, and a recent treatise under his editorship records present knowledge concerning actinomycin.[2] The cephalosporin C group of antibiotics was reviewed in detail by E. P. Abraham.[3]

Other reviews deal with recent advances in the chemistry and biochemistry of tetracyclines,[4] the chemical modification of antibiotics,[5] a survey of antibiotics in chemotherapy,[6] and the cell-free biosynthesis of actinomycins,[7a] tetracyclines,[7b] and streptomycin;[7c] further reports call attention to the role of variant bacterial forms and mycoplasmas in disease,[8,9,10] to the chemotherapy of enterobacterial infections,[11] and to the ecology of transferable drug resistance in the enterobacteria.[12] The judicious use of antibiotic combinations has been reviewed from the standpoint of a clinician.[13]

Abstracts[14] and a review[15] of a recent chemotherapy conference mirror current areas of prime interest; discussions of the optimal duration of antibiotic therapy and of antibiotic synergism and antagonism have been published.[16]

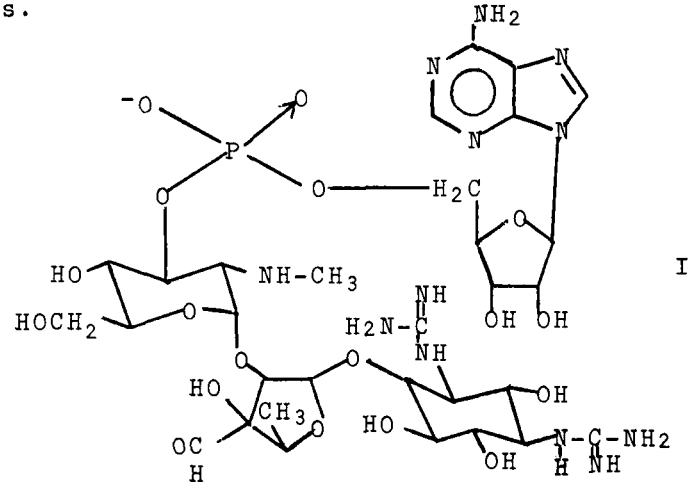
A group of antimicrobial polycyclic monocarboxylic acids, including monensin,[17a,b] the closely-related nigericin,[18] dianemycin, and others, are of fundamental biochemical and physiological significance because of their quite selective action on alkali metal ion-transport across natural (mitochondrial, red cell) membranes. Information concerning these ionophorous antibiotics[19a,b,c] as models for biological transport and concerning the related macrotetrolides[20] has been summarized.

Transmissible Drug Resistance. Reports [21-28] which describe mechanisms underlying bacterial resistance in exact enzymatic and molecular terms have added to our understanding of the provocative problem of "infective" drug resistance.

Inactivation of streptomycin,[26-28] kanamycin,[21,22,24] and the derived product paromamine[21,23,25] by an enzyme preparation from multiply resistant gram negative bacteria involves the site-specific adenylation or phosphorylation of the hydroxy group on C-3 of the N-methylglucosamine, the 6-amino-6-deoxy-D-glucose and the glucosamine moieties, respectively. This resistance factor (RF) mediated enzymatic inactivation process appears to be independent of the bacterial source. Thus, inactivated kanamycin-phosphate[24] obtained from the parent antibiotic by treatment with an enzyme from a resistant Ps. aeruginosa strain (H-9) was shown to be chemically identical with the product similarly obtained using an E. coli strain (ML 1629) carrying R-factor; such similarity was also observed for the enzymatic inactivation of paromamine by these two bacterial species.[25]

On the other hand, the phosphorylative inactivation of kanamycin by the RF-mediated resistant E. coli strain does differ from that involved in the resistance of laboratory acquired strains of E. coli 12.[21] The RF-mediated enzyme of E. coli (ML 1629) responsible for the inactivation is absent in sensitive E. coli 12 strains, strongly suggesting a direct relationship between this inactivating process and the observed resistance.[21] Adenylation as a means for the inactivation of streptomycin by RF-resistant E. coli has been reported from two additional laboratories.[27,28] Whether adenylation, rather than phosphorylation, is actually the first step of inactivation of aminoglycoside antibiotics other than streptomycin remains to be established.

The studies mentioned here have culminated in the detailed proof of structure of adenylylstreptomycin (I)[26,28] and may serve as a basis[27] for the design of biologically active, enzyme refractory analogs of aminoglycoside antibiotics.



I

A British report of a localized, high incidence of infantile enteritis,[29a,b] due to multiply resistant E. coli strains carrying R-factors, mentions the possibility of transfer of R-factors from man to man and, under predisposed conditions, from livestock to man. Analysis of the epidemiology of R-factors in Klebsiella infections in a Boston hospital allowed a definition of factors controlling spread (rare) to other bacterial species.[30] A rise of S. typhimurium infections in calves in Britain in 1964-66, attributed[29b] in part to the introduction of intensive farming methods using antibiotic feeds, prompted recommendations to minimize such risks. A thorough discussion of the current status of R-factors[31] lends perspective to these complex issues.

Other approaches to the proper assessment of R-factor effects include (a) a survey of the incidence in 1967 of antibiotic resistance in Salmonella from clinical sources in the United States[32] showing no significant increase during the past five years; (b) a study of the incidence of resistance (present) and transmissible factors (not present)[33] in drug-free primitive human and animal communities; (c) analysis of pre-antibiotic era isolates (some resistance);[31] and (d) the search for and the mode of action of agents (acridines) which prevent effective transfer of R-factors.[31] To facilitate such search, a model for the study of R-factor transmission in vivo has been reported.[34] Also, a thousand-fold reduction of frequency of R-factor transfer from donor to recipient strains of E. coli has been achieved by clinimycin at 15 µg/ml and by the highly active analog 7-deoxy-7(S)-chloro-N-demethyl-4'-pentyllincomycin at 4.0 µg/ml in vitro.[35]

The varied modes of antibiotic activity are presented in two reports which discuss the interaction of antibiotics with nucleic acid[36,37] and the inhibition of nucleic acid and protein synthesis.[38]

β-Lactam Antibiotics. The papers concerning penicillins and cephalosporins presented at a symposium in 1967 have been published in book form. The topics discussed include the chemistry of penicillins and cephalosporins in relation to biological activity,[39] total synthesis of β-lactam antibiotics,[40] and studies on the mechanism of action.[41]

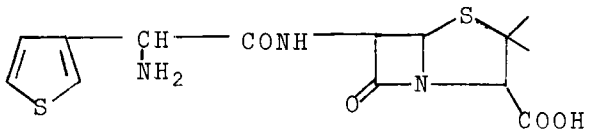
The presence in Penicillium chrysogenum of an acyl transferase which can catalyse in a cell-free system the transfer of the acyl group from acyl-coenzyme A to 6-APA has been demonstrated in several laboratories.[42,43,44,45] This was not a general acyl transferase, and a correlation of the specificity of the acyl group utilization in the 6-APA

transferase and in penicillin production by fermentation was found. No acyl-transfer activity was found in this enzyme system with penicillin N. The deacetylcephalosporin C in cephalosporin C fermentation broths results from a non-enzymatic, rather than an enzymatic, hydrolysis of cephalosporin C.[46]

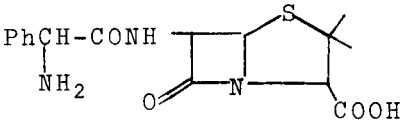
The relative importance of the various antigens, the antibiotic itself, degradation products, and impurities, that have been implicated in the allergic reaction to penicillin has been discussed by several authors.[47,48,49] Partial cross allergenicity between penicillin and cephalothin in humans has been demonstrated.[50,51]

Further clinical reports provide evidence for the utility of orally administered cephaloglycin in urinary tract infections.[52,53,54] Cephaloglycin, given orally, is metabolized by two pathways, deacetylation and side chain amide hydrolysis.[55] The deacetyl derivative of cephaloglycin has been obtained, and its properties recorded.[56] Cephalexin continues to have promise as an effective oral broad spectrum cephalosporin antibiotic.[57,58] Results obtained in rabbits and monkeys demonstrate the nephrotoxicity of cephaloridine when given in large doses and the lack thereof with cephalothin at the same dose level.[59]

A number of new derivatives of 6-APA and 7-ACA have been made and evaluated. Of particular interest is 6-(R-α-amino-3-thienylacetamido)penicillanic acid (II) whose *in vitro* antibacterial activity is comparable with the clinically useful ampicillin (III) but is significantly more active in animal protection tests when administered orally.



II

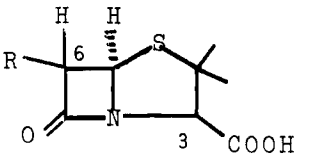


III

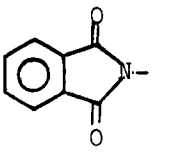
Results indicate that the thienyl derivative is better absorbed orally.[60] Series of substituted sydnone-carboxamido-[61] and -acetamido-[62] penicillanates and cephalosporanates have been prepared. Various substituted isothiazole carboxamido-[63] and acetamido-[64] penicillanates and cephalosporanates have also been studied. Several members of the two general series have antibacterial activities comparable to clinically useful, semi-synthetic penicillins and cephalosporins.

The syntheses of monocyclic[65,66] and bicyclic[67,68,69] analogs of penicillin have appeared; however, no significant antibacterial activities have been reported.

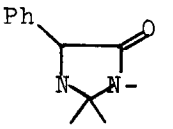
Isomerization of penicillins at C-6 has been studied by several groups of workers. Methyl 6-phthalimidopenicillanate and hetacillin are epimerized by base to the respective derivatives, IIIa (as the methyl ester)[70] and IIIb.[71]



III a. R =



b. R =

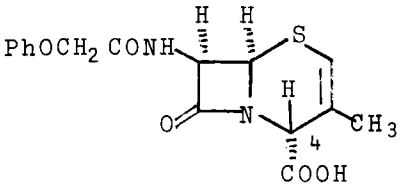


c. R = NH_2-
d. R = $PhCH_2CONH$-
e. R = $PhCHCONH$-
NH_2

From the IIIb the epi-6-APA (IIIc),[72] epi-Pen G (IIId),[72] and epi-ampillicin (IIIe)[73] have been obtained. Compounds IIId and IIIe show very low antibacterial activity in comparison to materials having the natural configuration at C-6. The 6-epi-penicillin V methyl ester has been synthesized.[74] The aldol condensation of formaldehyde and benzaldehyde on carbon 6 in 6-APA has been studied,[75] and the 6-hydroxybenzyl penicillin G synthesized from the latter showed only weak antibacterial activity.

An interesting rearrangement product has been obtained from nitrosation of 6-APA,[76] and a related substance from treatment of 6-chloropenicillanic acid with nucleophiles.[77]

The catalytic reduction and desulfurization of a Δ^2-cephalosporin derivative to a dethiopenicillin, obtained also from penicillin V, indicates that the configuration at C-4 in IV is the same as at C-3 in penicillins.[78] Details of a



IV

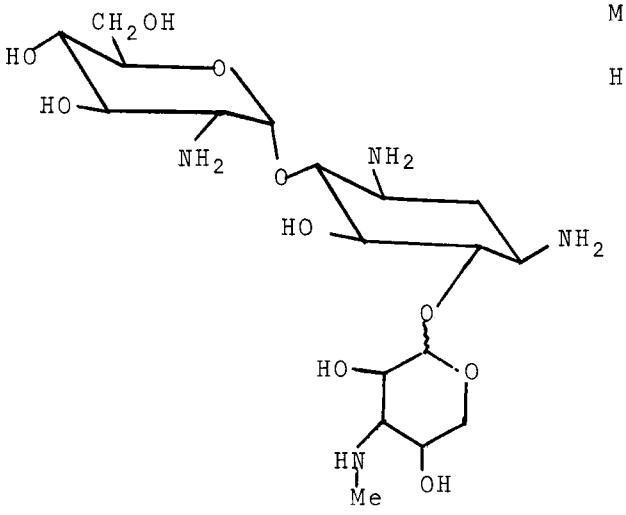
practical chemical cleavage of cephalosporin C to 7-ACA, involving the conversion of a protected cephalosporin C to an intermediate imino-chloride and imino-ester, have been reported.[79] The reaction of aromatic amines on cephalosporanic acids has been studied,[80] and one product obtained resulted from a transformation similar to the conversion of penicillanic acids into derivatives of penicilloic acid amides.

Aminoglycosides. This class acquired increased importance in 1968 with gentamycin sulfate (Garamycin®) becoming available to the physician for the intramuscular treatment of infections due to susceptible strains of gram-negative bacteria, including Pseudomonas aeruginosa. Clinical trial reports[81a,b] involving 150 patients showed gentamycin or combinations of gentamycin and cephaloridine to be more effective than existing agents (kanamycin, colistin, polymyxin B or combinations thereof) in the initial therapy of serious acute gram-negative rod infections prior to cultural definition. The superiority of gentamycin in reducing mortality rates was particularly evident in cases of more severe illness and in a lower frequency of nephrotoxicity (6%) per group of patients than was seen with kanamycin (21%), polymyxin B (20%) or colistin (13%).

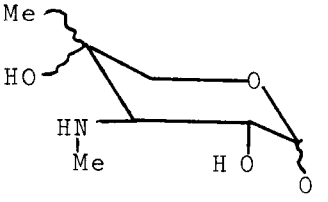
Reports of additional clinical trials[82,83] have appeared and reviews of the antibacterial[84] and pharmacological properties[85] are available. Changes in the acoustic system in the guinea pig showed gentamycin at the clinically employed dose levels to be appreciably less ototoxic than streptomycin.[86] Urinary tract infections in children with gram-negative organisms showing in vitro resistance to other available drugs were effectively treated with gentamycin.[87] Gentamycin ointment has been used successfully in burn wound sepsis as an adjunct to the systemic administration of the antibiotic for the control of Pseudomonas infection.[88]

Pure, crystalline gentamycin A has been prepared[89] and was shown to possess structure V in which the known paromamine (= pseudoneamine) is connected through C_6 of the 2-deoxystreptamine moiety to C_1 of a novel 3-methylamino-3-deoxy-

pentopyranose designated gentosamine.[89] The closely-related garosamine (VI)[90] was recognized as a fragment common to the individual C_1, C_2, and C_{1a} components[91] of the gentamycin C complex; the remainder of the molecules consists of the three gentamines which contain 2-deoxystreptamine linked to purpurosamines A, B, and C respectively.



V



VI

The total synthesis of kanamycin A from suitably protected 2-deoxystreptamine has been accomplished[92] by successive coupling with multiply blocked 6- and 3-amino-glucopyranosyl chlorides followed by deblocking steps. The synthetic kanamycin A possessed the expected activity against *E. coli* and *B. subtilis* cultures. The total syntheses of kanamycins B[93] and C[94] as well as A[95] have been achieved recently. A series of N, N', N", N'''-tetraphenylalkyl-kanamycin derivatives[96] were prepared, some of which inhibited kanamycin-resistant *E. coli* and *S. aureus* as well as sensitive strains. Chemical modification of kanamycins[97] to give, for example, 3-amino-3-deoxykanamycin, coupled with inspection of natural analogs[98] augments our understanding of structure-activity relationships among aminoglycoside antibiotics.

The total synthesis of kasugamycin in stereochemically pure form has been achieved, albeit in low yield;[99] in its biosynthesis, glycine-N has been shown to be incorporated into the unique carboxyformidoyl function[100] of this inositol-glycoside. The chemistry and biochemical mode of action of streptomycin was reviewed.[101] Analysis of concentration effects on the extent of misreading of RNA code words induced

by various aminoglycosides suggests that some members operate by a "one site," others by a "multiple site" contact at the ribosome.[102]

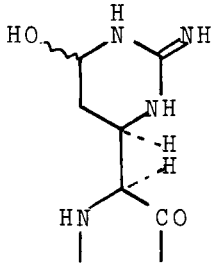
Peptide Antibiotics. Capreomycin has been evaluated in combination with PAS,[103] with PAS and isoniazid,[104] and with ethambutol,[105] in the treatment and re-treatment of chronic pulmonary tuberculosis; as a result the use of capreomycin in multiple drug combinations has been advocated for the treatment of infections due to isoniazid resistant tubercle bacilli.[105]

Viomycin has been shown to inhibit protein synthesis[106] but not to cause codon misreading in the bacterial ribosomal system. Thus, the base properties of antibiotics alone seem not to be enough to cause miscoding, which confirms the notion that streptamine or deoxystreptamine moieties of aminoglycoside antibiotics are essential for miscoding activity.

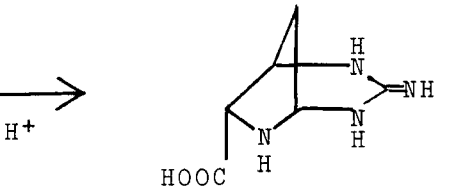
The unsettled state of viomycin chemistry is being resolved.[107-111] Structure (VII) proposed[107] in 1966 for viomycidine has received strong support from chemical studies[100,111] and has been confirmed by X-ray analysis;[112,113] viomycidine is now thought[110] to be an artifact generated during acid treatment from fragment (VIII) in viomycin. Dihydroviomycidine (Xa) obtained from hydrogenolyzed viomycin (fragment IX) was shown to be identical with capreomycidine[108,111] (derived directly from capreomycin) and apart from its optical behavior to be identical with the synthetic racemate of α-(2-iminohexahydropyrimidyl)glycine[109] (Xb). Dihydroviomycidine (Xa) is not a product of hydrogenation of viomycidine (VII).[108]

The elusive chromophore (λ max 268 mμ; ε = 24,500) of viomycin and, by analogy, that of capreomycin has now been formulated[110] as the structural unit (XI) and appears to be unrelated to the guanidine-fragment (VIII). The total structure linking fragments VIII, XI and amino acids[110,114] remains to be established. Stendomycin is discussed elsewhere in this Section, Chapter 13.

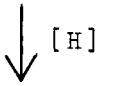
The elucidation of the structure of telomycin,[115] a macrocyclic peptide lactone, discloses the presence of three novel amino acids, erythro-β-hydroxyleucine, cis- and trans-3-hydroxy-proline; the unusual chromophore in this antibiotic (λ max 339 mμ; ε = 22,000) is another example of a dehydroamino acid (dehydrotryptophan) unit (XII).

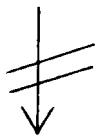


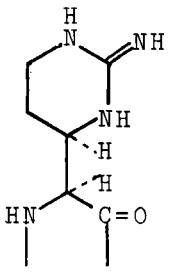
VIII



VII







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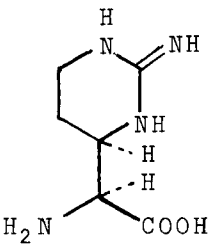
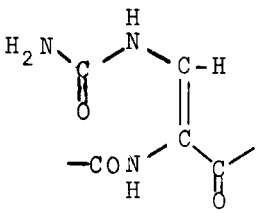
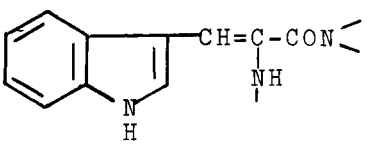


Xa
Xb, racemate



XI



XII

A generalized biosynthetic theory for peptide antibiotics envisages that the formation of macro-ring systems involves the insertion of amino acids into smaller units, e.g., diketopiperazines and postulates that the formation of D-amino acid constituents takes place from L-amino acids after their incorporation into stereochemically labile intermediates.[116] There seems to be general agreement that the biosynthesis of peptide antibiotics, "even" gramicidin,[117] is a purely enzymatic process and does not depend on ribosomal-RNA participation required in protein synthesis.

Tetracyclines. Minocycline, 7-dimethylamino-6-demethyl-6-deoxytetracycline, is effective orally at 1-10 mg/kg against tetracycline-resistant staphylococcal infections in mice which fail to respond to 1000 mg/kg of tetracycline.[118] Several clinically isolated strains of Neisseria meningitidis proved sensitive to minocycline--and to doxycycline--at <0.78 mcg/ml, which suggests consideration of these antibiotics as candidates for prophylaxis in meningococcal outbreaks.[119] Minocycline at 100 mg/kg/day in a drug diet is effective against M. tuberculosis H_{37Rv} infections in mice[118] while doxycycline and tetracycline are inactive at 400 and 600 mg/kg respectively; ethambutol is effective at 50 mg/kg.

Clinical reports which describe the favorable response of urinary tract infections[120] as well as staphylococcal pyoderma and pneumococcal pneumonia[119] delineate the merits of minocycline in relation to existing broad-spectrum antibiotics.

A comprehensive report[121a,b,c] on antimicrobial, pharmacological, and clinical qualities of doxycycline (Vibramycin®) relates the relatively high lipophilicity of doxycycline to the rapid attainment of adequate blood levels of 1-3 mcg/ml in man after single oral doses of 50-250 mg;[121b] the calculated half life was 15 hours.[121b] Also, doxycycline penetrates and concentrates in localized areas of inflammation in levels which make effective antimicrobial chemotherapy possible.[122] In an extensive trial among 81 patients suffering from sepsis, bile duct, urinary and pulmonary infections due to gram negative and positive organisms, 42 (51%) became and remained bacteria-free; side effects were insignificant.[121c] The susceptibility of bile duct infections to doxycycline has been confirmed.[123]

A complete review of tetracycline biosynthesis[124] includes mention of the intriguing non-occurrence of 5-oxychlortetracycline which is, nevertheless, readily formed from its presumed precursor, 5a (11a)-dehydrochlortetracycline, by cell free or whole cell S. rimosus preparations.

The successful total synthesis of the major antibiotic terramycin[125] constitutes a versatile two-stage process which augments previous approaches and may afford novel members of this therapeutic class.

Chloramphenicol. Amidst dock repairs and new navigational instructions for the diagnostuary, the tides of flood and faster craft abreast, and overhead "dear captain" broadcasts for selection of the shipwrecked, the veteran lifeboat still picks them up at sea.[126] Strongly cautioning against inadequate dosage and duration, against the possible emergence of

resistant strains and of resistance transfer factor,[127] a recent editorial[128] restates the need for chloramphenicol as drug of choice in the treatment of the vulnerable patient with Salmonella gasteroenteritis.

A chloramphenicol-resistant S. aureus as well as enteric bacteria carrying R-factor inactivate the antibiotic[129,130] through conversion to the 3-O-acetyl- or 1,3-O,O-diacetyl derivative by chloramphenicol-acetyltransferase with utilization of acetyl-CoA. The 3-deoxy analog of chloramphenicol, which is itself neither an effective antibiotic nor a substrate for the transferase, was found to be a potent inducer of the enzyme.[131]

Chloramphenicol inhibits protein synthesis; why it does not do so in the case of S. venezuela which produces it is a question raised in a review of its biosynthesis.[132] Similar bacteriostatic activity of the same order against S. aureus was found among the four optical isomers of threo-2-dichloroacetamido-1-(4-methylsulfinylphenyl)-1,3-propanediol;[133] the para CH_3SO-substituent renders these analogs inferior to chloramphenicol itself.

Clinimycin. 7(S)-Chloro-7-deoxylincomycin is superior[134,135] to lincomycin in that it shows significantly greater anti-staphylococcal and -pneumococcal activity and that its absorption is not decreased when taken with meals; doses of 75 to 150 mg afford assayable blood levels for 12 to 24 hours. Clinical usefulness has been demonstrated in bacterial pneumonia, group A streptococcal pharyngitis and tonsilitis, and against other gram positive infections.[136,137] The surprise activity of the chlorolincomycins against Plasmodium berghei infections in mice is maintained against chloroquine and diaminodiphenylsulfone resistant strains.[138]

Ansa Macrolides. Rifampicin's (rifampin) potential in the treatment of pulmonary tuberculosis has been further strengthened by laboratory[139] and clinical[140-142] investigations while its merit in the therapy of staphylococcal[143] and other infections[144] remains to be established.

While several prominent antibiotics do affect protein synthesis at the level of the bacterial ribosome, the rifamycins,[145-148] streptovaricins[149a,b] possibly other ansa macrolides, e.g., tolypomycin,[150] in cell-free extracts as well as "in vivo"[148] specifically inhibit DNA-dependent RNA polymerase of sensitive--but not of resistant[149b]--B. subtilis and E. coli. Unlike actinomycin, which is known to inhibit this enzyme in the mammalian cell through binding to template DNA, the ansa macrolides do so through the formation

of a stable complex with the bacterial enzyme[147] and, as a consequence, even at higher levels do not inhibit DNA-directed DNA synthesis.[145] Finally, that the rifamycins fail to inhibit RNA polymerase of mammalian origin[146] may be reflected by the favorable activity-to-toxicity ratio of this novel type of antibacterial.

Structure-activity studies in the rifamycin group deal with the detrimental effect of the carboxyl function,[151] and the beneficial effect of acetylation of hydroxyl groups in the aliphatic connecting chain.[152]

The structure determinations of streptovaricins A and C,[153] and of tolypomycin,[149] ansa macrolides which resemble rifamycin S in their gross structure and in the nature of their aromatic chromophore, have been successfully accomplished. An oxygenated cyclobutane ring is part of the connecting chain in the former two, while tolypomycin, in addition to a cyclopropane ring, features an aminosugar, tolyposamine, condensed at the quinonecarbonyl-carbon.

Macrolides. Clinical papers report the use of spiramycin in the effective prevention of post-operative staphylococcal infections in the elderly,[154] and possibly of congenital toxoplasmosis in pregnancy.[155] Enhancement of erythromycin activity against gram-negative bacteria has been noted upon alkalinization of the assay medium or the urine.[156]

Specific binding of erythromycin, spiramycin, and of lincomycin to the 50S ribosomal subunit of sensitive B. subtilis appears to be the intracellular event responsible for cell growth inhibition by these agents.[157] Such binding results in the suppression of protein synthesis by interference with the attachment of peptidyl-tRNA to the ribosome.[158] The cell-free protein synthesizing system of E. coli is also inhibited signifying that lack of inhibition of gram-negative cell growth by these agents must be due to other reasons, for example, the observed 100-fold decrease of cellular drug accumulation.[159] Ribosomes from resistant B. subtilis under the same conditions fail to bind erythromycin.[160]

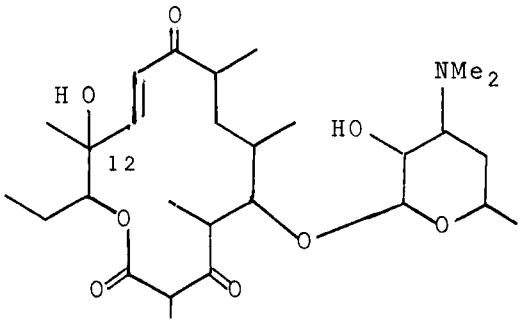
The intriguing proposition has been made that the macrolide glycosides as well as lincomycin are "recognized" by ribosomal receptor-sites as resembling the terminal unit of peptidyl-[159] or aminoacyl-tRNA.[160]

Continued studies of erythromycin biosynthesis utilizing partially blocked S. erythreus mutants have resulted in the isolation of 6-deoxyerythronolide B shown to be an intermediate in the erythromycin pathway immediately preceding erythronolide B;[161] another isolate, 5-deoxy-5-

oxoerythronolide B was recognized as a shunt metabolite.[162] The biosynthetic pathways for branched sugars occurring in macrolides have been surveyed.[163]

The pyrolytic elimination of N,N-dimethylhydroxylamine from the N-oxides of erythromycins A and B afforded the corresponding allylic alcohols, 3'-de(dimethylamino)-3',4'-dehydroerythromycins A and B;[164] these elegant reactions provide a novel, gentler procedure for degradation studies.

The structures of leucomycin A_4-A_9 have been shown to be C_2-C_5 fatty acid esters replacing the isovaleryl group on mycarose in A_3;[165] the availability of these and of semi-synthetic analogs made it possible to relate their structure and lipophilicity with biological activities.[166] The structure of picromycin has been thoroughly revised[167,168] to the 14-membered diketonic lactone (XIII) which differs from narbomycin only by the presence of the tertiary alcohol group at C_{12}.



XIII

Boromycin, $C_{44}H_{72}O_{15}NB$, contains boric acid held in a tetradentated complex with the aid of valine and a poly-hydroxy-compound, $C_{39}H_{66}O_{14}$, presumably a macrolide; it constitutes a first naturally occurring, well-defined boron compound.[169]

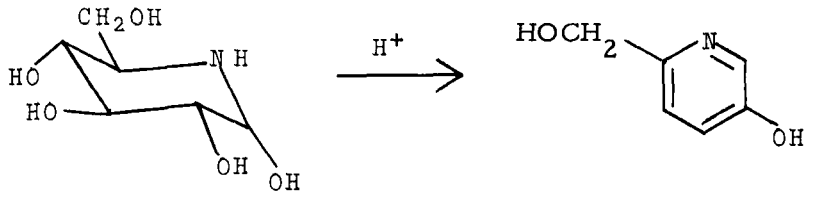
Miscellany. Sensitivities of oral microorganisms to vancomycin[170] and the effective control by topical vancomycin of necrotizing gingivitis[171] have been described. Vancomycin and ristocetin form a complex with mucopeptide precursors of several bacteria.[172] The former agent inhibits cell wall biosynthesis with the accumulation of UDP-peptides; the inhibition of cell growth is preventable by cell walls of sensitive bacteria.[173]

Attention is directed to the inhibition of cell wall biosynthesis by enduracidin,[174] a basic polypeptide, and by the phosphorus containing antibiotics moenomycin[175] and

prasinomycin.[176] Reportedly, a single dose of prasinomycin protects mice against Gram-positive infections for as long as a month.[176]

The semi-synthesis of about a hundred coumermycin A_1 analogs using novobiocin fragments has afforded some potent antibacterials[177] for evaluation.

Difficulties in the isolation of nojiromycin,[178] a labile antibiotic with appreciable activity against S. lutea, were admirably surmounted. This permitted the determination of its unusual structure as 5-amino-5-deoxy-D-glucopyranose (XIV). Mild acid converts XIV to 5-hydroxy-2-pyridylcarbinol (XV).



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Chapter 10. Synthetic Antibacterial Agents

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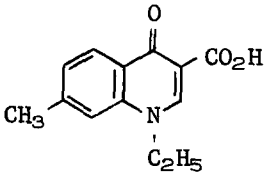
Introduction - In the pattern now characteristic of this mature field, advances have come this year in new versions of established agents or in a consolidation of knowledge about them. The emphasis in this report has been placed on in vivo activity as it has been in previous years; but, as in previous years, the vast and important field of topical agents has not been neglected.

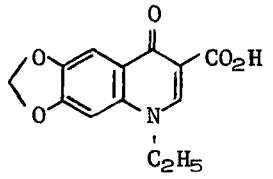
Reviews - The experimental aspects of the chemotherapy of tuberculosis were reviewed by Batten,[1] clinical aspects were discussed editorially.[2] Browne reviewed the treatment of leprosy.[3] A favorable editorial comment was made on the use of an antimutagen (quinacrine) with standard treatment of urinary tract infection to prevent the rise of resistant bacterial strains.[4] Lampe examined the changing concepts in the therapy of urinary tract disease.[5] The not unrelated fields of sulfonamides,[6] and nitrofurans[7] were also reviewed. The resistance development as a consequence of antimicrobial drugs in animal feeds was examined.[8] Topical antimicrobials were reviewed in several contexts; antiseptics and disinfectants,[9] soap bacteriostats,[10] deodorants and antiperspirants,[11] and antimicrobial biguanides.[12] The topical treatment of burns and the renaissance of mafenide for such use was discussed by Moncrief.[13] Veterinary ophthalmic antimicrobials were reviewed.[14]

Quinolone Antibacterial Agents

Mode-of-Action - Overlooked in the 1967 review was a paper by Boyle, Goss and Cook on nalidixic acid I; an alteration of normal DNA replication (in E. coli) occurs under its influence. In the new pattern, DNA synthesis occurs at new sites on the chromosomes, and requires concomitant protein and/or RNA synthesis.[15] Pianotti, Mohan and Schwartz implicated DNA metabolism in the mechanism of action of the related oxolinic acid II.[16]

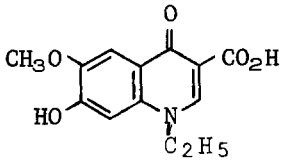


nalidixic acid I



oxolinic acid II

Derivatives - The synthesis and activity in vitro and in vivo against Proteus vulgaris was reported for II and 28 allied compounds.[17] The presence of a free carboxyl, ethyl (or substituted ethyl) on the ring nitrogen and an oxo function are important for high activity. Metabolic disruption of the methylenedioxy ring of oxolinic acid II led to a new derivative III which was isolated from the urine of healthy volunteers who had been dosed with II.[18]

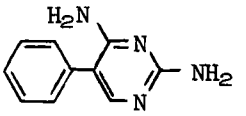
III 

Therapy of Urniary Tract Infections - Nalidixic acid I was compared with sulfisoxazole in two groups of 15 patients (30 total) treated for 10 days. Sulfisoxazole was more effective in the control of these infections.[19]

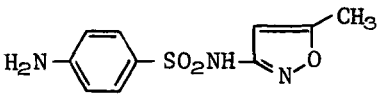
Sulfonamides

New Derivatives - Sulfadiazine disubstituted in the pyrimidine ring with 6-methyl-5-methoxy was effective, disubstituted with 6-methoxymethyl 5-methoxy was ineffective, when tested against S. pyogenes in mice.[20] Active N-sulfanilylcytosines were described.[21]

Trimethoprim IV, a Sulfa Potentiator - After about a decade since its first introduction trimethoprim IV has arrived at a stage of active clinical testing. A flurry of papers on its laboratory and clinical testing appeared this year. The practical use of an antifolic sulfa synergist is old in parasitic diseases but new in bacterial infections particularly with Gram negative infections. The laboratory aspects of its action were



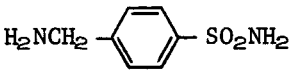
trimethoprim IV



sulfamethoxazole V

recapitulated by Bushby and Hitchings.[22] They reported that IV binds bacterial dihydrofolate reductase significantly greater than earlier analogs in the chemical series with no increased binding of mammalian enzyme. The sulfa synergism is shown in vitro and in vivo. In clinical studies trimethoprim IV has been paired with sulfamethoxazole V which shows the same excretion pattern (half-life of 14 hours).[23] The drug (IV) has a mild inhibitory effect on folate metabolism in man at the tissue level which can be counteracted by administering folinates.[24]

Sulfonamides for Burns - An old remedy has been revived for this use. The drug is mafenide VI, a sulfonamide whose action is not reversed by PABA. The high levels reached by this soluble substance applied topically allow inhibition of bacteria not usually sensitive to sulfonamides. Moncrief



mafenide VI

has reviewed this development.[13]

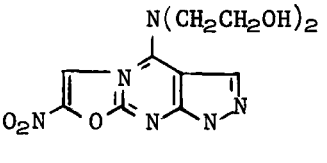
Unrelated is the advocacy of the insoluble silver sulfadiazine for burn treatment because of its great action against Pseudomonas.[25]

Physicochemical and Metabolic Aspects - Extensive studies of binding to bovine serum albumin with theoretical interpretations were made by Moriguchi and coworkers.[26,27] A quantitative study of the effect of binding on activity was given by Anton.[28] Physicochemical factors governing the excretion of sulfonamides were studied and correlations with pH, binding and partition coefficients were tested.[29]

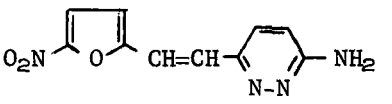
Therapeutic Aspects - Already cited in this review is the study which showed sulfisoxazole superior to nalidixic acid in urinary tract infection.[19] The news was released that FDA sponsored studies showed generic sulfisoxazole forms reached peak levels more slowly and were lower than the brand name drug Gantrisin®. The differences were thought not to be medically significant.[30]

Nitrofurans and Allied Antibacterials

New Derivatives - Burch described the synthesis and activity of 52 nitrofurylpyrazolopyrimidines.[31] The most active, and representative, was VII which was effective against S. aureus mouse infections at about 2 mg/kg; it also was active in vitro. Activity was widespread among closely

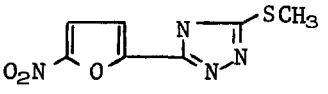


VII



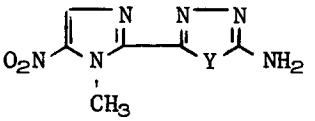
VIII nifurprazine

related derivatives. A new nitrofuran, nifurprazine VIII was given clinical trial as a topical agent in Germany; the study involved 2000 patients.[32] A series of nitrofuryl and nitrothienyl amidines and imino ethers and their triazole cyclization products with semicarbazide and thiosemicarbazide was investigated.[33] Most active was IX.



IX

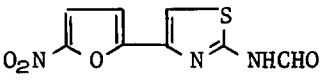
For this field the outstanding announcement was that nitroimidazole can replace nitrofuran to form potent broad spectrum antibacterials. The original announcements were a series of papers presented at the Eighth Interscience Conference on Antimicrobial Agents and Chemotherapy in October. Some of this work has since been published by Berkelhammer and Asato and will be cited here.[34] Best activity was exhibited by X with



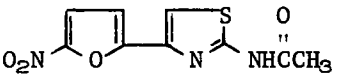
X, Y = S or O

the thiadiazole being preferred. The compounds have high activity against Salmonella, E. coli and Pasteurella multocida in chicks and mouse infections. In addition, in the mouse, activity was shown against Neissera, Klebsiella, Aerobacter, Shigella, Streptococcus and Staphylococcus species. No chronic toxicity testing was cited.

The derivative XI produced urinary bladder carcinoma in rats. The related compound XII which has been used in Europe to treat urinary tract infections has not been reported to be carcinogenic but is under further test for chronic toxicity.[35] (This report appeared in 1967 but was not in last year's review.)



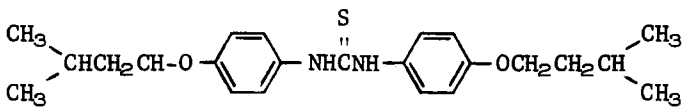
XI



XII

Tuberculosis

Established Drugs - The oxidative degradation of ethionamide to sulfoxide and other products in vitro was studied with relation to activity in tuberculosis.[36] A test for ethionamide sulfoxide in urine was devised so that clinicians could check that patients were ingesting the drug.[37] Clinical and laboratory experience with ethambutol was reported in 15 papers and was summarized by Herman.[38] The pro and con of the isoniazid prophylaxis of tuberculosis was discussed,[39,40,41] and the production of hepatic carcinoma in mice with this agent was disclosed.[42] The metabolism of doubly labeled thiocarlide XIII was studied in humans.[43] Blood levels were 10-12 mcg/ml from doses 6 g per day, twice the level seen in rabbits.



thiocarlide XIII

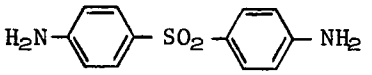
Mechanism of Action - Experiments were performed which supported the view that the activity of isoniazid comes from a conversion intracellularly to isonicotinic acid, a similar conversion for ethionamide accounting for its action also. The isonicotinic acid(s) thus liberated intracellularly would otherwise not have gained access to the cell.[44]

New In Vitro Active Hydrazine Compounds- Tuberculostatic activity was reported for the following: five different dextran bonded isoniazids,[45] hydrazones, semicarbazones and thiosemicarbazones of 2-pyrrolealdehyde and of dihydroxyalkylbenzaldehydes,[46] acylated isoniazid, phenylsemicarbazides and thiosemicarbazides,[47] 2-(p-acetamidophenyl)-4-benzylidine-5-oxazolone was reacted with INH to give straight chain derivatives with up to 100 times the activity INH,[48] hydrazides and hydrazones from diphenylsulfone acids,[49] and aryl hydrazides of disubstituted glycolic acids.[50]

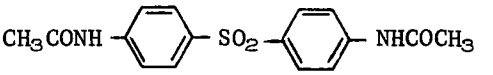
Miscellaneous In Vitro Active Compounds - Significant activity was shown by 3-[p-(isopentyloxy)phenyl]-6-hydroxy-4(3H)-quinazolinone and some related compounds,[51] 6-(hexanoylthio)purine (25 mcg per ml),[52] and benzothiazolylamidines.[53]

Leprosy

In his review on leprosy Browne[3] asserts that dapsone XIV is the drug of choice for treatment of the disease and discusses alternate chemotherapeutics. Hastings and Trautman[54] described the use of dapsone combined with streptomycin in the treatment of relapsed lepromatous lep-



XIV

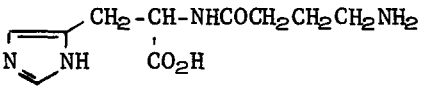


XV

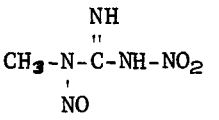
rosy. Shepard, Tolentino and McRae[55] reported on the successful treatment of leprosy with diacetyldapsone XV given as a repository intramuscular dose, 225 mg, once per 77 days. By several objective criteria the therapy by this regimen matched that of dapsone, XIV, given perorally daily at 100 mg per day. Glazko, Dill and Montalbo[56] describe an extremely sensitive fluorometric method for the determination of dapsone XIV and acetyl derivative XV in blood and tissue. The half-life of XV in an intramuscular dose in oil is about 42 days by their determination. Certain substituted azonaphthylamines showed in vivo activity in rat leprosy.[57]

New In Vivo Active Antibacterials

Homocarnosine XVI was highly effective against Staph. aureus infections in mice.[58] The nitrosoguanidine derivative XVII had activity against Staph. aureus and E. coli infections in mice.[59]

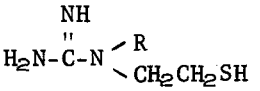


homocarnosine XVI



XVII

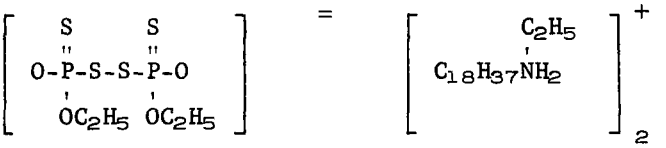
High in vitro activity and some in vivo activity (Staph. aureus in mice) was shown by the mercaptoethylguanidines XVIII.[60]



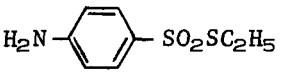
XVIII

Antiseptics, Topical Agents and Soap Bacteriostats

Antibacterial Action of Established Agents - Extensive testing in the USSR of the topical antifungal and antibacterial aminophosphone XIX was reported; it has been used topically on over 450 patients with good results.[61]

XIX 

Also in USSR the manufacture and clinical use of esulan XX has been approved. The compound has bacteriostatic and fungistatic properties.[62]

XX 

Observations were reported on the following classes related to known antibacterials: biguanides,[63,64] chlorinated diphenyl ethers,[65] and chlorophenoxycarbanilides.[66]

Mechanism of Action - Hamilton proposes that 3,5,3',4'-tetrachlorosalicylanilide acts at the cell membrane with prior penetration of the cell wall to the membrane as a necessary first step; at the membrane energy dependent transport is inhibited. This mechanism is shared by a wide variety of topical agents.[67] The role of association with proteins in the mechanism of action of phenolic disinfectants was the subject of another report.[68] Lien and associates correlate antibacterial activity with partition coefficients; two characteristic coefficients were found, one for Gram negative and another for Gram positive cells.[69] Increasing lipid content in Staph. aureus increasingly protects them against lipophilic phenols.[70] The genetic determinants for drug resistance were eliminated from E. coli by sodium dodecylsulfate, the survivors were

drug susceptible.[71] The binding of proflavine to transfer ribonucleic acid was demonstrated.[72]

Miscellaneous Compounds of Unknown In Vivo Potential - Antibacterial activity was reported for the following heterocycles: 3-substituted benzoxazolinones,[73] N^6-alkyladenines,[74] halogenated benzimidazoles,[75] diaminopyridopyrimidines,[76] dihalobenzotriazoles,[77] a mercaptopyrimidothiazole,[78] methoxyquinazolines,[79] diarylisoxazoles,[80] arylpyrazolones,[52] oxirenes,[81] and phenyl pyridyl ethers.[82] Active aromatic and aliphatic compounds were: (α-aminobenzyl)phosphonic esters,[83] substituted β-aminoketones,[84,85] adamantaneamides,[52] 3-decynoyl-N-acetylcysteamine,[86] a silyl compound,[87] and an acetylenic mercapto ester.[86]

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Chapter 11. Antiviral Agents

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Background information on antiviral agents was covered in a general overview of antiviral drugs[1], a report on the effect of 156 biologically active compounds against influenza and vaccinia infections in mice[2] and a survey of antiviral agents for 1967[3]. In addition, several short reviews were concerned with the control of specific virus diseases by various means including the use of antiviral agents and interferon[4,5,6,7]. One report, although not specifically concerned with antiviral agents, discussed factors in the host-virus relationship which could affect the disease process as well as response to therapy[8].

Adamantanes and Other Amines - Continuing challenge and field trials with 1-adamantanamine HCl (amantadine HCl; "Symmetrel") not only confirm the protective efficacy of the drug against influenza A2 infections of man and animals but also indicate therapeutic activity. In one series of double-blind placebo-controlled tests a total of 58 volunteers were treated with 100 mg of amantadine HCl or placebo twice daily starting 26 hours prior to infection with influenza A2/Rockville/1/65 and treatment was found to significantly diminish the occurrence and severity of the induced disease[9]. In other studies human volunteers were challenged with influenza A2/Moscow/21/65, A2/Leningrad/133/67 or A2/Hong Kong/1/68. Preliminary reports indicated that prophylactic treatment with 100 or 200 mg of amantadine HCl per day provided a good degree of protection against the disease which was greater than obtained with vaccine. In addition, it was also reported that drug plus antibody or immune γ-globulin provided an additive effect in these challenge studies[10]. In January 1968 amantadine HCl was used in a therapeutic study in the U.S.A. during an influenza A2 outbreak. A total of 96 men, considered to have clinical influenza of short duration and a fever of at least 37.8°C (100°F) were treated with 200 mg per day of drug or with placebo for 10 days. The amantadine HCl-treated patients showed a more rapid alleviation of illness than did the placebo patients and no drug-associated reactions were reported[11]. During the 1967-1968 winter another therapeutic study was carried out in Japan by the Research Committee on Antiviral Agents. Three hundred and fifty-five volunteers were placed in the placebo-controlled study with drug at 200 mg/day for adults and at 50 to 150 mg/day in children, depending on age. The results were similar to the previous study with a statistically significant reduction in duration of fever and maximum temperature in the amantadine HCl-treated groups and with no drug-associated reactions[12]. Amantadine HCl was tested for the prevention of upper respiratory disease in a group of 96 placebo-controlled volunteers. Although treated and placebo groups had the same incidence of upper respiratory disease, the drug-treated subjects were reported to have a lower incidence of hoarseness and fever as well as less discomfort from coughing[13].

Laboratory experiments with mouse-adapted influenza A2/Taiwan/9/64 in mice indicated that either vaccination or amantadine HCl treatment provided protection from death and that combined treatment showed an additive effect[14]. In another laboratory study it was demonstrated that although amantadine HCl treatment offered a high degree of protection against death in mice infected with influenza A2/Japan/305/57, treatment did not significantly reduce lung titers nor reduce the transmission of infection to recipient mice[15]. This was in contrast to treatment with α-methyl-1-adamantanemethylamine HCl (EXP 126; rimantadine HCl) which caused a reduction in all three parameters. Therapeutic activity of amantadine HCl was also demonstrated in mice by oral administration of drug started twelve hours after illness was apparent following infection with influenza A2 strains, as evidenced by a reduction in drinking water intake[16]. Although treatment of influenza A2 infections in man and animals generally results in a lowered antibody response, this does not appear to be a direct effect since continuous oral or single IP treatment caused no effect on antibody response to phage T_2 in mice[17].

Tissue culture studies have shown an additive effect from combined interferon and amantadine HCl treatment of chick embryo cells infected with influenza A/WSN[18]. Amantadine HCl has also been shown to be effective in tissue culture against strains of influenza A2 isolated in Japan in 1957, 1964 and 1965 and influenza A1 isolated in 1953 but not on a 1964 influenza B strain[19]. Classical fowl cholera was sensitive to amantadine HCl in tissue culture[20] while preliminary studies indicate that oral doses of amantadine HCl reduce mortality from fowl plague in turkeys[21] and influenza infections in quail[22].

α-Methyl-1-adamantanemethylamine HCl, previously mentioned as causing a reduction in lung titer, spread of virus to recipient mice and mortality of mice infected with influenza A2/Japan/305/57[14], was studied in a single placebo-controlled influenza A2/Rockville/1/65 challenge study in 56 volunteers at 400 mg/day. The drug-treated group showed a significant reduction in amount and severity of disease compared with the placebo group[23].

Cyclooctylamine HCl demonstrated antiviral activity against a variety of myxoviruses as well as IBR, herpes zoster and vaccinia in tissue culture and against influenza A2/AA/2/60 and A/Swine/S15 but not A/PR8 in mice[24]. Other amine and ammonium compounds were found to be effective against influenza A viruses in mammalian cells as measured by a quantitative immunofluorescent technic[25] with a mechanism similar to that of amantadine HCl.

Isoquinolines - 1-(p-Methoxyphenoxymethyl)-3,4-dihydroisoquinoline HCl (UK 2371), 1-(p-chlorophenoxymethyl)-3,4-dihydroisoquinoline HCl (UK 2054) and 1-phenoxymethyl-1,2,3,4-tetrahydroisoquinoline (UK 2792) were found to have antiviral activity against a number of different viruses in tissue culture and the chick embryo. Against some of the sensitive strains the compounds acted as virus inactivators; against others there was an antiviral effect without inactivation[26,27]. In a series of challenge studies

with influenza A2/Leningrad/4/65 and B/England/101/62 in small groups of volunteers of unknown antibody status, UK 2371 at relatively high doses (1.5 grams daily for 7 days) was reported to produce a statistically significant reduction in antibody rise and virus isolation, but not in reduction of symptoms in the influenza B challenge group. The influenza B test groups were combined for analysis although the infecting viruses in the trial series ranged from virulent man-to-man passage virus to twenty-egg-passage virus. No detectable side effects were noted. However, neither hematological nor biochemical tests were performed. UK 2054 was stated to produce a similar, though non-statistically significant, response against influenza B infection[28].

Extensive laboratory studies indicated that 3,4-dihydro-1-isoquinoline acetamide (DIQA) possessed antiviral activity against both DNA and RNA viruses, a spectrum not usually found with nontoxic compounds. Infections of mice with herpes simplex, Columbia SK, ECHO 9 and influenza A and B strains responded to intraperitoneal (IP) or subcutaneous (SC) treatment of nontoxic concentrations of DIQA[29].

Nucleosides - Laboratory data on 9-β-D-arabinofuranosyladenine (Ara-A) was extensively discussed in a series of reports[30,31,32,33,34,35]. Ara-A was effective against herpes simplex infections of mice by IP, SC, oral and topical treatment started either prior to or after infection. Topical treatment with Ara-A of scarified hamsters eyes infected with herpes simplex prevented death and healed the eyes. Although high doses of compound were necessary, the material had a low order of animal toxicity with no mortality in mice at 7,950 mg/kg orally and an IP LD_{50} of 4,677 mg/kg. In tissue culture antiviral activity was reported against strains of adeno, cytomegalo, vaccinia, varicella and human and simian strains of herpes viruses. The topical activity and low toxicity of Ara-A indicate it is a promising candidate for treatment of herpes infections.

5-Iododeoxyuridine (idoxuridine; IUDR; "Stoxil"; "Herplex") was administered intravenously to a newborn infant with diagnosed systemic herpes infection. Clinical improvement was noted for a period but was followed by relapse and death[36]. Topical application of IUDR dissolved in dimethylsulfoxide or systemic treatment with IUDR was reported to accelerate healing of herpes simplex lesions on guinea pigs, but without eliminating virus[37].

5-Bromo-2-deoxyuridine and pyran copolymer were used to treat four infants with subacute sclerosing panencephalitis with some evidence of arrest of disease but no reversal[38]. Studies in HeLa cells infected with herpes simplex virus and treated with 5-fluoro-2'-deoxyuridine (FUDR) suggest that the residual virus found after treatment may be due to recoating of input virus DNA without actual synthesis of new viral DNA[39].

Thiosemicarbazones - 1-Methylisatin-3-thiosemicarbazone (methisazone; "Marboran") prevented but did not cure smallpox in man and had a favorable effect on eczema vaccinatum and vaccinia gangrenosa. A wider range of activity was seen in tissue culture with adeno, polio, varicella

and foot-and-mouth disease virus reported as sensitive[40]. Challenge infections in man with type III adenovirus did not respond to preventative or curative treatment with methisazone or trifluorothymidine[41] in spite of laboratory indications of sensitivity. 1-Methyl-3-(4,4-dibutylthiosemicarbazone)indole-2,3-dione (busatin) was reported to inhibit polio virus replication as well as RNA synthesis in tissue culture[42]. Two strains of rhinoviruses, HGP and 1059, were reported to be inhibited in a plaque test by a number of thiosemicarbazones, biguanides and S-triazines although most of the compounds were also cytotoxic[43].

Other Antiviral Compounds - Many members of the picornavirus group are sensitive to the activity of 2-(α-hydroxybenzyl)benzimidazole (HBB) in tissue culture. Representatives of the 55 numbered serotypes of the rhinoviruses were checked for inhibition by HBB and the more active D(-)HBB·HCl, and it was concluded that in spite of variation in degree of inhibition, sensitivity to D(-)HBB·HCl appeared to be a characteristic of this group[44]. Three strains of lymphocytic choriomeningitis were inhibited by HBB in HeLa cells, but unlike the picornaviruses were insensitive to guanidine[45]. Activity in tissue culture against polio, Coxsackie, ECHO, herpes simplex and neurovaccinia virus has been reported for a number of optical isomers and N-alkylated derivatives of 5-chloro HBB with greatest activity found for the 1-propyl analog[46]. Other tissue culture studies indicate anti-poliovirus activity for a number of 1,2-bis(2-benzimidazolyl)-1,2-ethandiol derivatives[47]. 2-Diethylaminoethyl 4-methylpiperazine-1-carboxylate has shown activity against influenza infections of mice and nineteen of a series of analogs also have anti-influenza activity but none was more active than the parent compound[48].

Topical treatment with α-keto-β-ethoxybutyraldehyde (kethoxal) was reported to cause a favorable response in herpes simplex infections of baby rabbits and hairless mice with the suggestion that this agent could have potential utility in man[49].

Tissue culture experiments have shown antiviral activity for tetramethyldipicrylamine (TMP) against myxovirus, picornaviruses and group A arboviruses[50] with antiviral activity also reported for 2-(pyridyl)-benzthiazole analogs against influenza A/PR8 and parainfluenza 1 in chick embryo chorioallantoic membranes[51].

Antiviral Agents from Natural Products - Following the report of antiviral activity of gliotoxin, a fungus metabolite, in 1966 extensive studies were carried out on this agent and analogs. Gliotoxin blocked virus-induced RNA synthesis but not cellular RNA synthesis in tissue culture[52]. The fungus Arachniotus aureus produced a number of related compounds with antiviral activity[53,54] with aranotin and acetylaranotin the most important. The structure of acetylaranotin (LL-S88a), when produced by Aspergillus terreus, has been determined[55,56]. Aranotin was highly active (0.12-1.0 μg/ml) in tissue culture[57]. LL-S88a inhibited rhino, Coxsackie, polio and parainfluenza viruses in tissue culture as well as protected mice from lethal infections with Coxsackie A21 or influenza B/Maryland[58].

Unfortunately these compounds show antiviral activity *in vivo* only by parenteral but not by oral administration[59]. A number of simple analogs based on the common epidithiopiperazinedione nucleus have been synthesized and several have shown tissue culture but no *in vivo* activity against Coxsackie and rhinoviruses[60].

Other biological products with reported antiviral activity include: phagicin, a virus protein from λ-infected *E. coli* cells with tissue culture and *in vivo* activity against vaccinia and herpes simplex[61]; aqueous extracts of giant puffballs and the black Japanese forest mushroom with activity against influenza A2/Japan/305/57 infections of calf kidney cells and influenza A/PR8 infections of mice[62]; and hot water extracts of the bark of the Japanese medicinal plant *Sambucus sieboldiana* which reduced mortality of mice infected with Columbia SK and LCM virus[63].

Interferon - A large volume of laboratory studies on interferon and interferon inducers was published in 1968, but not matched by clinical reports. Although many but not all viruses have the ability to stimulate interferon production in animals[64,65], this survey will be largely limited to work on nonviral inducers. Up-to-date reviews include a discussion on the potential usefulness of the interferon mechanism[66], a discussion of our knowledge to date on interferon[67] and a survey of advances in 1967[3].

Administration of exogenous interferon is reported to exert a protective effect in tissue culture against adeno-2 virus[68] and in mice has prolonged life or prevented death caused by Rauscher virus[69], vesicular stomatitis virus[70] and encephalomyocarditis virus[71]. There are reports that some interferons could cross species lines and be effective[72,73] unlike earlier evidence of greater specificity.

In spite of the efficacy of exogenous interferon, the major search has been for interferon inducers. Synthetic polymers have been effective inducers and some of the characteristics apparently necessary for activity are as follows: a large molecule with a high density of free anionic groups, not readily degradable and not readily eliminated. Agents with these characteristics include polymers of maleic anhydrides, polyacrylates and carboxylates[74]. Polyacrylic acid (PAA) and polymethacrylic acid (PMAA) have induced interferon in tissue culture and protected mice infected with Mengo virus[74] and vaccinia and vesicular stomatitis virus[75,76]. Salts of polyvinyl sulfate appear to have dual activity since they induce interferon activity in mice but not in tissue culture, and the antiviral activity exerted is not correlated with virus interferon sensitivity[77].

Double-stranded nucleic acid polymers have been demonstrated to be highly effective interferon inducers. Polyriboinosinic-polyribocytidylic acid complexes, but not the individual homopolymers, induced interferon in a number of cell lines[78] as was the case for a synthetic double-stranded DNA polymer[79]. Severe herpes simplex keratoconjunctivitis of rabbit eyes was reported to be cured by treatment with a double-stranded polyinosinic:polycytidylic RNA[80]. Treatment was effective when started up to

three days after infection thus showing curative activity and indicating that interferon treatment need not only be prophylactic. Further evidence has been presented that the activity of statolon is due to a double-stranded RNA from a virus infecting the fungus[81,82]. MM virus infection of mice responded to treatment with 1 μg of this material and statolon has also been reported to prolong the life of mice with established Friend leukemia virus[83] as well as to protect mice infected with lethal aerosols of Columbia-SK virus[84]. High levels, 5-20 mg, of a double-stranded DNA protected mice with lethal infections of EMC virus presumably due to an interferon mechanism[85]. One study with double-stranded synthetic polynucleotides in mice suggested that the mode of action was a release of preformed interferon rather than the induction of new material[86]. Among agents with a reported adverse effect on interferon production are certain chronic virus infections[87], dl-19-nor-D-homotestosterone[88], serotonin[89] and an unknown factor in sera of various species[90].

Screening Methods and Rapid Diagnosis - The search for more effective screening methods continues. Among newer methods proposed was the use of virus isolation in the chimpanzee for testing rhinovirus leads[91] and virus isolation in nonlethal HA-1 virus infections of the hamster[92]. Other parameters of nonlethal infections included measuring the decrease in water intake of influenza-infected mice[16] and decrease in weight gain of Coxsackie-infected mice[93]. Another system of interest was the use of an animal model to study the transmission of influenza virus infections[94,15]. At the tissue culture level quantitative hemadsorption was suggested as a fast and reliable test for anti-myxoviral agents[95].

Rapid diagnosis methods have largely been concerned with immunofluorescent technics and recent tests include those for influenza A/PR8[96], respiratory syncitial[97], adenovirus[98] and a more generalized sandwich technic for myxoviruses[99]. One novel technic described the use of gas chromatography of serum samples to detect new components formed by the virus infection[100] in animals which has the potential of rapidity and specificity.

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Chapter 12. Human Antiparasitic Agents

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Introduction - A comprehensive review of current therapy and advances in human parasitism is provided in several publications which have appeared during the past year.[1-9] A possible new approach to the treatment or prophylaxis of malaria, toxoplasmosis and other protozoan infections, is the use or induction of interferon.[10-15] Killed bacteria or bacterial endotoxin is reported to stimulate resistance to malaria.[16,17] The emergence in man of intestinal capillariasis (capillaria philippinesis), a new parasitic illness capable of reaching epidemic proportions, presents a new challenge in antiparasitic chemotherapy.[18-21] Another area of growing importance is the increased incidence of amebic meningoencephalitis in diverse areas. This lethal disease is attributed to the presence of free-living ameba of the genera Naegleria or Hartmannella.[22-27] An axenic culture medium for Naegleria has recently been developed.[28] At least three species of Hartmannella were isolated from throat swabs in routine examinations of "normal" persons.[29] Returnees from Vietnam and other parts of the globe are contributing to the rising incidence of parasitic infections in the United States.[30-32] World-wide, it has been estimated that about 200 million people suffer from schistosomiasis, 450 million from ancylostomiasis, 190 million from filariasis, 50 million from onchocerciasis, 650 million from ascariasis and 7 million from Chagas' disease.[33]

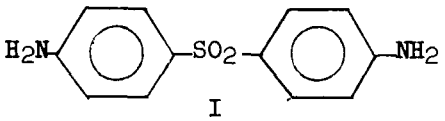
PROTOZOAN INFECTIONS

Malaria - Progress and status of the current World Health Organization program on malaria and other parasitic diseases is reviewed in the 1968 WHO Chronicle.[33,34] Automated in vitro approaches to the screening of potential antimalarial drugs are being explored.[35-39] For the most part, the studies are concerned with the effect of drugs on the metabolism of parasitized red cells. The implications of the monkey in the transmission of simian and human malarias are under study.[40,41]

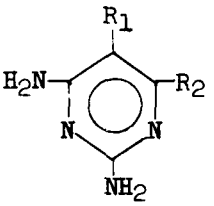
Current Chemotherapy - The antimalarial chemoprophylactic regimen involving the weekly use of chloroquine (300 mg. base) and primaquine (45 mg. base) is still employed in Vietnam. Medication is continued on a weekly basis for eight weeks after returning to the United States. The daily use of dapsone (I) (25 mg.) in addition to the above regimen as a prophylactic against chloroquine-resistant strains of P. falciparum is being investigated.[32]

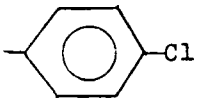
For the treatment of malaria, 600 mg. (base) of chloroquine is given as the initial dose followed by 300 mg. (base) six hours later and then 300 mg. base daily for two days. In conjunction with chloroquine, 15 mg. (base) of primaquine is administered daily for fourteen days to eliminate the exoerythrocytic forms of the parasite.[32] If the disease does not respond rapidly to the above treatment, or if chloroquine-resistant

falciparum is suspected, 650 mg. of quinine sulfate is given orally three times daily for ten to fourteen days together with pyrimethamine (IIa) 25 mg. twice a day for three days and dapsone (I) 25 mg. per day for 28 days.[32,42] A combination of chloroquine, pyrimethamine and sulfisoxazole may be equally effective.[43]



I



a) R_1 = 

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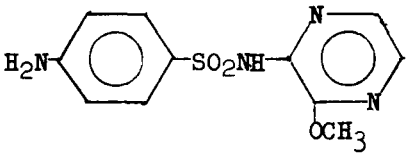

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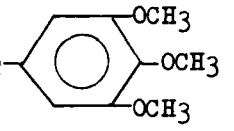
b) $R_1 = CH_2$ 

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II

Studies with a combination of trimethoprim (IIb) and sulfalene (III), which showed encouraging results against 4-aminoquinoline-resistant malaria, are continuing.[44] Other combinations of antimalarial drugs are also being investigated.[1,6,45-56] Primaquine may play an important role in preventing transmission of the resistant P. falciparum.[57]

The widespread contributions leading to the solution of the problem of resistant malaria[1,58] may be our most significant victory in Vietnam.[59]

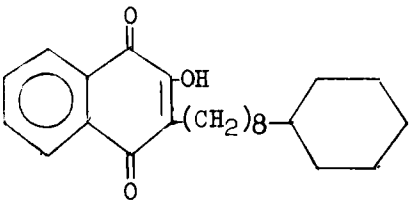
Modes of Action - There is a renewed emphasis on the study of the metabolism of the malarial parasite, its interactions with drugs and the effects of the parasite on the host metabolism. These investigations are reviewed in a recent symposium.[60-66] The inhibition of nucleic acid biosynthesis by antifolic compounds and by DNA polymerase inhibition is stressed. Morphological changes in the malarial parasite are associated with drug effects on metabolism.[67-73] The role of PABA may extend beyond its importance as a folic acid precursor. Combinations of trimethoprim, which inhibits dihydrofolate reductases, with sulfonamides, which are PABA blockers, have been shown to have significant antibacterial as well as antimalarial activity.[1,59,74,75] The high activity and favorable picture regarding development of resistance make these combinations very attractive.

The binding of 8-aminoquinoline antimalarials to DNA comparable to that with chloroquine has been reported.[76] The effect on DNA function may be involved with antimalarial activity. Co-enzyme Q which is associated with the mitochondrial oxidation of DPNH and succinate and is present in the metabolism of the parasite has been shown to be inhibited by chloroquine, primaquine, quinacrine and menoctone (IV), thus suggesting diversity of action for antimalarial drugs.[77]

Potential Chemotherapeutic Agents - Interest continues with chloro-

lincomycin derivatives (V) which have recently been reported to be effective against P. cynomolgi in rhesus monkeys[78,79] as well as against P. berghei in mice.[80] Menoctone (IV) was recently reported to have causal prophylactic activity against sporozoite-induced P. berghei infections.[81]

A large number of new 2-phenyl-4-quinolinemethanols (VI) have been synthesized in an effort to eliminate the phototoxicity in man associated with this group. Chlorine atoms have been replaced by fluorine atoms in the phenyl and quinoline rings,[82,83] the 2-phenyl group has been replaced by CF_3[84] and the side chain S has been changed to include α-heterocyclics.[85,86] Many of the above compounds, as well as an α-(alkylaminomethyl) (S = $CH_2NHC_4H_9$)[87] were reported to be active against P. berghei; however, the phototoxicity problem has not been overcome.



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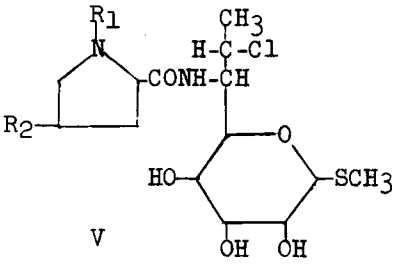

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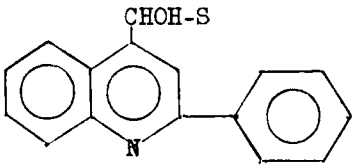

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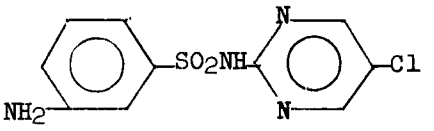


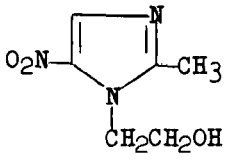
VII

Included among the various antimalarial compounds synthesized which show activity in mice are: 6,8-disubstituted pyrido [2,3-b] pyrazines,[88] mono- and di-sydnone derivatives of DDS,[89,90] 3-phenylrhodanines,[91] guanylhydrazone salts of aromatic ketones,[92] 4-aminobenzo-[g]-quinolines[93] 2-amino-1,4-naphthoquinoneimines,[94] 2-(aminoalkylamino)-4-(trichloromethyl)-s-triazines[95] and p-sulfamoylphenylazo compounds.[96]

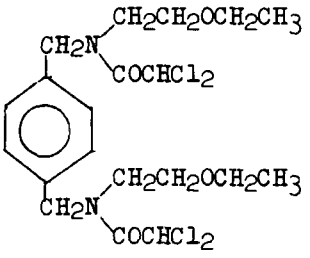
Metachloridine (VII) has been reexamined and shown to be active in rodent malaria. It is less active than some of the newer sulfonamides, but is considerably less toxic.[97] The phenazine derivative, 2-(4-chlorophenyl)-3-isopropylimino-5-(4-chlorophenyl)-3,5-dihydrophenazine, which has been shown to have antileprosy activity in man, has now been found to be active against P. berghei.[98] Antipantothenates are added to the list of substances reported to be effective against avian malaria, but which lack appreciable effects on P. berghei in mice.[99]

The large number of publications which have appeared during the past year is an indication of the renewed interest and importance of the malaria problem.

Amebiasis - The anti-amebic properties of metronidazole (VIII) both in intestinal and hepatic infections have been demonstrated. The results suggest that a high concentration of the drug is more important than duration of treatment. Doses of 1.2 - 2.4 grams per day for five days were effective.[100] Teclozan, a bis(dichloroacetamido) compound (IX) which is used for intestinal amebiasis, has been investigated in a mental hospital as a prophylactic agent. In a six-month study, a weekly dose of 200-300 mg. resulted in a reduced reinfection rate compared to a control group of untreated patients.[101]

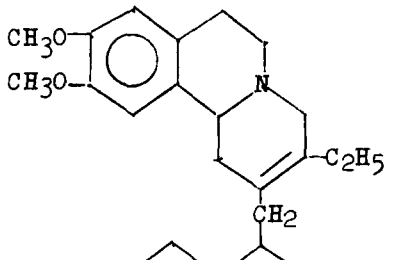


VIII

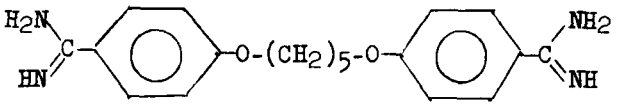


IX

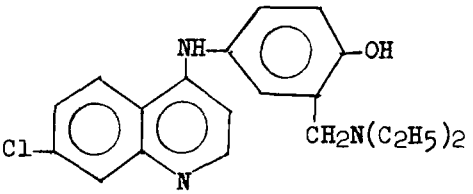
There are conflicting reports on the use of oral dehydroemetine (X) in amebic dysentery.[102,103] Although it appears to be less cardiotoxic than emetine, its use in extra-intestinal amebiasis is still limited.[103,104] In intestinal amebiasis, a two-day treatment of approximately 2 grams of paromomycin per day is as effective as a five-day course.[105] A controlled-release preparation of erythromycin stearate used in the treatment of intestinal amebiasis was shown to produce very low blood levels and high fecal concentrations. Clinical and parasitological cures were obtained in 60%-90% of the patients.[106-108] Contrary to the accepted view, the halogenated 8-hydroxyquinoline derivatives are absorbed in considerable amounts from the intestine and, therefore, may not be innocuous.[109,110] In vitro studies show antiamebic activity for some 2,6-disubstituted-4-chloropyrimidines[111] and styrylimidazole derivatives.[112]



X



XI

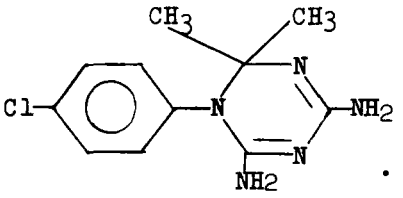


XII

Miscellaneous - Pentamidine (XI) is effective in the treatment of Pneumocystis carinii pneumonia, a serious disease which has been found in pat-

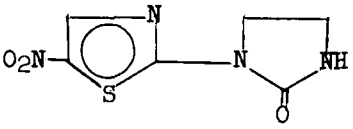
ients receiving immunosuppressive therapy (for leukemia, lymphoma, connective tissue disease or transplant rejection).[113-117] An experimental model of pneumocystosis in cortisone treated rats is available.[118] New nitroimidazole derivatives have been reported to have antitrichomonal activity.[119-121] A single dose of amodiaquin (XII) (600 mg.) shows promise in the treatment of giardiasis.[122]

Several recent reports have attested to the effectiveness of single intramuscular injections of cycloguanil pamoate (XIII) in the treatment of cutaneous leishmaniasis.[123-126] Metronidazole (VIII)[127] and pentamidine (XI)[128] have also been used with some success. Oral activity of [N-(5-nitro-2-thenylidene)-3-amino-5-(N-morpholinyl) methyl-2-oxazolidone] in hamsters and mice against visceral leishmaniasis has been demonstrated.[129]



· $C_{23}H_{16}O_6$

XIII



XIV

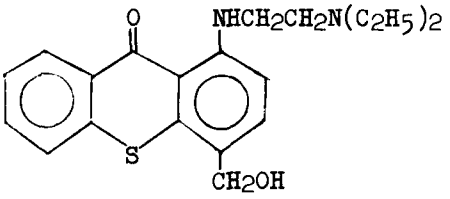
METAZOAN INFECTIONS

Schistosomiases - The estimated world-wide distribution of schistosomiasis, the populations exposed and the morbidity and mortality rates in various countries are included in a recent publication of a symposium on schistosomiasis.[130] The estimated incidence of this disease, which is believed to be a conservative one, is approximately 118 million. Other estimates are as high as 200 million.[131] The ultimate eradication of schistosomiasis may rest in mass chemotherapy coupled with mollusc control.[132-133]

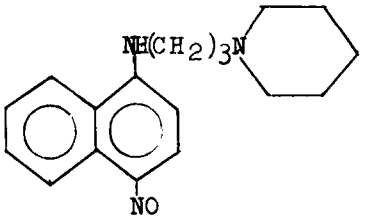
A survey of current therapy and modes of action with schistosomicides has been published.[1,3] Niridazole (XIV), which is particularly effective against S. haematobium, is not recommended for mass treatment of schistosomiasis because of hepato-splenic involvement.[134]

Potential Chemotherapeutic Agents - New reports on the use of hycanthone (XV) by oral and intramuscular routes in the successful treatment of S. mansoni have been published.[135-138] A recent paper[139] describes results with hycanthone in 97 patients infected with S. mansoni or S. haematobium or both. Oral medication doses of 2.5 mg/kg/day for 3 or 4 days or a single intramuscular injection of 3.0 or 3.5 mg/kg were given. After a follow-up of three months, the clearance rate for both routes of administration ranged from 80-94%. Several patients who were cleared at three months passed viable ova at six months. These were considered as possibly due to reinfections. The high activity and fewer and milder side effects with the single injection could be of great advantage for mass chemotherapy. Hycanthone is not the metabolite of lucanthone which appears to be involved in antileukemic activity.[140] On this basis, there is no corre-

lation between schistosomicidal and antitumor activity.



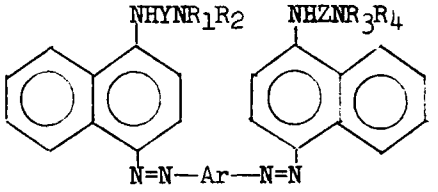
XV



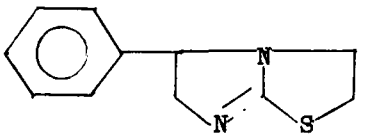
XVI

Daily treatment (60 mg. by s.c. injection) with dehydroemetine (X) for periods of 1-3 months was effective in eliminating eggs from urine and stools in 33 patients with S. mansoni.[141] In a series of nitrosonaphthylamines, compound (XVI) was found to be highly effective against S. mansoni in mice and monkeys, but suffered from a relatively narrow therapeutic index.[142] Some bis(4-arylazo-1-naphthylamines) (XVII) have also been reported active against S. mansoni in mice.[143]

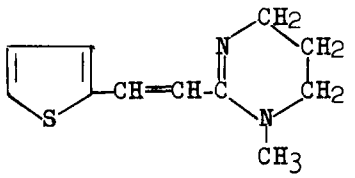
Nematode Infections - There have been several review articles dealing with chemotherapy of nematode infections.[4,5,7,9] Reports on tetramisole (XVIII) have appeared confirming its activity against ascarid infections in man.[144-149] As indicated earlier,[1] treatment of other helminth infections was not as satisfactory.[150-152] A new synthesis of tetramisole starting with the condensation of styrene oxide and ethylenimine has been reported.[153]



XVII



XVIII



XIX

$(CH_3O)_2POOCH{=}CCl_2$

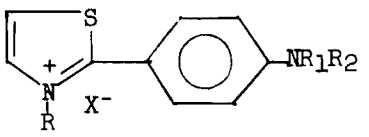
XX

Preliminary results of clinical trials with the pamoate salt of pyrantel (XIX) against intestinal nematodes are encouraging. Cures of 93-98% were reported in two antipinworm studies (79 patients) using single doses of 5 and 6 mg/lb. of the drug (as base). A slightly higher dose gave 93% cures (49 patients) of ascarids and 82% cures (40 patients) of hookworms.[154] Both tetramisole and pyrantel are reported to inhibit cholinesterase.[155]

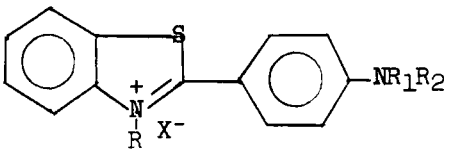
Good results were reported for dichlorvos (DDVP) (XX) given in a resin formulation.[156,157] Doses of 6 mg/kg and 12 mg/kg were effective against hookworm, ascarid and trichurid infections. Apparently, the dosage form is important in preventing serious anticholinesterase activity of this organophosphate.

Several papers have been published dealing with the anthelmintic activity of the di-isothiocyanate (XXI). The compound appears to be effective against hookworm[158-163] and ineffective against ascarids.[159,160] Niridazole (XIV) in 25-30 mg. daily doses for ten days was effective in the treatment of guinea-worm infections (Dracontiasis).[164,165] Animal studies with thiazolinium (XXII)[166] and benzothiazolinium salts (XXIII)[167] indicate antinematode activity.

XXI



XXII



XXIII

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Chapter 13. Antifungal Agents

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The use of such medicinal and therapeutic agents as broad spectrum antibiotics, antitumor agents, steroids, immunosuppressants, x-irradiation, and oral contraceptives have been reported to contribute to an increased incidence of superficial, cutaneous, or deep-seated mycoses.[1-6] The frequency of fungal infections complicating acute leukemia in the period from 1959 to 1964 doubled when compared with the frequency of the preceeding 5-year period.[7,8] While agents such as griseofulvin, tolnaftate, pyrrolnitrin, and nystatin offer adequate control of the superficial and cutaneous mycoses, there has been only moderate success in treating the systemic fungal infections with new agents such as saramycetin and 5-fluorocytosine. To the present time, the polyene antibiotics have been the most clinically useful chemotherapeutic agents for deep-seated mycoses. Although amphotericin B has been used widely in human medicine, disadvantages such as poor oral absorption and nephrotoxicity make this antifungal antibiotic far from the ideal drug.[9,10] Therefore, the need exists for additional safe and effective antifungal agents.

Griseofulvin - The oral use of micronized griseofulvin is highly recommended for treatment of Tinea capitis (ringworm of the scalp).[11,12] Based on observations of 128 patients, griseofulvin therapy (1 g per day) was indicated for Tinea cruris, Tinea unguium, and Tinea capitis; this antibiotic had limited utility in the treatment of other Tineas caused by species of Trichophyton.[13] A correlation of Trichophyton rubrum resistance to griseofulvin with clinical failure has been observed.[14] A 5% griseofulvin ointment was a useful topical treatment for many dermatophytoses; however, for scalp and nail infections systemic treatment should always be combined with local therapy. This antibiotic was reported to be a successful treatment of Raynaud's disease, an uncommon skin malady in children.[16]

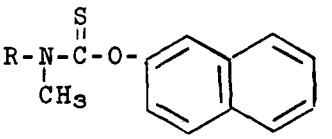
Further reports have appeared indicating that griseofulvin is a well-tolerated drug even in patients with general or systemic diseases as well as in individuals of advanced age.[17] However, contraindications which have been reported include Candida infections, Tinea versicolor, erythrasma, and porphyria.[18] Studies concerned with porphyrin excretion in 29 adults and 13 children receiving griseofulvin showed moderate though inconsistent increases of fecal protoporphyrin without altering porphyrin metabolism to any marked degree.[19] Evaluation of a series of griseofulvin analogues for their

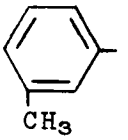
porphyria-inducing activity in chick embryo liver cells suggested that this antibiotic induces porphyria[20] by a mechanism unrelated to its antifungal action.[21] In the rat, rabbit, and dog, griseofulvin exerted a direct action on the cardiovascular system which was not mediated through neurogenic or hormonal mechanisms.[22]

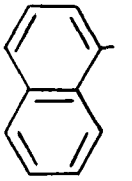
Following a single oral dose of 500 mg, average human plasma levels of griseofulvin were 1.18 μg/ml after 2 hrs which decreased to 0.86 μg/ml after 25 hrs.[23] Excretion studies of griseofulvin during a 24-hour period showed that 77% of an intravenous dose was found in the bile and 12% in urine of the rat; a reverse relationship between excretion in bile and urine was found for the rabbit; 4-demethylgriseofulvin and 6-demethylgriseofulvin were the dominant metabolites in bile and urine of both animals.[24]

The effects of griseofulvin on fungal morphology, cytology, respiration, cell walls, chemical composition, uptake of labeled substances as well as effects on absorption, translocation, metabolism, and reversal of inhibition have been summarized.[25] The stimulation of DNA synthesis by griseofulvin together with the formation of abnormal cells indicates that the mechanism of action of this antibiotic is associated with the replicating system of the fungal organism.[26] Interestingly, griseofulvin was an effective metaphase-arresting agent of oocytes of a marine annelid causing a rapid and reversible dissolution of the mitotic spindle.[27] However, the antibiotic inhibited binding of colchicine, a known metaphase arrestor, in disrupted tumor cells.[28]

Tolnaftate - A series of antifungal compounds called naphthiomates were described by Noguchi et al. in 1962.[29] These compounds exhibit a narrow but extremely potent antifungal spectrum. Two of the substances, naphthiomate T (I) and N (II), have received detailed evaluation.[30] The former, known as tolnaftate, is available as a 1% Polyethyleneglycol solution (TINACTIN™) for topical treatment of dermatophytoses.[31-33]



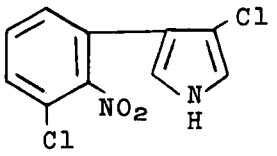
I; R = 

II; R = 

Recently powder[34] and cream[35] formulations have also been shown to be effective against various Tinea infections. Effective response to tolnaftate by patients with erythrasma, a disease recently considered to be of bacterial origin, has been used as evidence for the fungal nature of the causative organism.[36] While still primarily of interest as a topical agent, oral administration of 450 mg/kg of tolnaftate was as effective as 120 mg/kg of griseofulvin in the treatment of experimental Trichophyton infections on guinea pigs.[37] This study showed that tolnaftate is largely excreted in the feces with little absorption occurring. Small quantities of N-methyl toluidine and β-naphthol were detected in blood, urine, and feces.[37] The mode of action of this synthetic antifungal agent remains to be determined.

Detailed analyses of the change in antifungal activity with chemical modification have appeared.[38,39] Naphthiomate-N is very active, but the other three naphthalene isomer combinations are inert. Various substituted phenols may be used in place of the β-naphthol moiety. Of the N-methyl (essential for activity) anilines tried, hydrogen, methyl, methoxyl, and halogen containing compounds were active; whereas nitro, dimethylamino, carboxyl, etc., derivatives were inactive. The carbamates still retained some activity; but other variations of the thiocarbamate system, such as the dithiocarbamate derivatives, were devoid of antifungal action.

Pyrrolnitrin - A unique, substituted 3-phenyl pyrrole derivative was reported by Arima[40] and co-workers in 1964. This compound, produced in the fermentation of several species of the genus Pseudomonas,[41,42] was found to have structure III.



III [3-(2'-nitro-3'-chlorophenyl)-4-chloropyrrole]

Since that time, several halogen variations of the original compound have been isolated as minor co-produced metabolites.[43-46] Pyrrolnitrin is not only a potent inhibitor of dermatophytes, but also shows moderate activity against yeasts and a number of fungal plant pathogens and gram-positive bacteria.[47] The compound is highly effective, as a topical preparation, in the treatment of superficial dermatophytoses in animals and man.[47-49] Pyrrolnitrin has been available in Japan for clinical use since 1966 as PYROACE™.

An elegant synthesis of pyrrolnitrin has been

reported.[50,51] An α-blocked pyrrole ring was first synthesized onto 2-nitro-3-chloroacetophenone. This product was chlorinated at the 4-position, and methyls were oxidized in one step using sulfurylchloride; decarboxylation afforded authentic pyrrolnitrin. As the voluminous patent literature indicates, (inter al. [52,53]) this synthesis yields many new phenyl pyrroles for evaluation as antifungal substances. A comparison of the activity of close relatives of pyrrolnitrin has not appeared to date.

The biosynthetic origin of pyrrolnitrin has been studied.[54] Since the yield of this antifungal agent is increased when D-tryptophan is added to pyrrolnitrin-producing cultures, this amino acid appears to be a specific precursor. Incorporation of ^{14}C-labeled tryptophan yielded pyrrolnitrin containing 18% of the radioactivity.[45] As the level of added tryptophan increases, 3-chloroindole and 3-(2'-amino-3'-chlorophenyl)-4-chloropyrrole were observed. This latter compound, when reintroduced into a fresh culture, is rapidly converted to pyrrolnitrin. Excess 3-chloroindole added to the culture inhibits production of the antifungal substance.

New phenyl pyrroles are produced[55] when certain analogues of tryptophan (dl-6-fluorotryptophan and dl-7-methyltryptophan) are added to pyrrolnitrin-producing cultures. The new compounds, 4'-fluoropyrrolnitrin and 3'-methyl-3'-dechloropyrrolnitrin, still possessed significant antifungal activity. The x-ray crystal structures of pyrrolnitrin and 4-fluoropyrrolnitrin have been determined.[56,57]

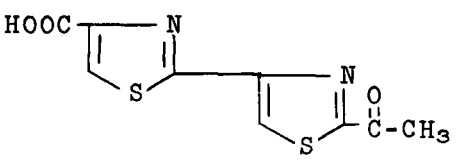
β-Phenyl pyrrole shows only marginal antimicrobial activity.[47] Several substitutions on the phenyl ring improved activity with m-trifluoromethyl phenyl pyrrole being most active.

A brief report on the mode of action of pyrrolnitrin indicated that the primary action was on the cell membrane, resulting in cellular permeability impairment and leakage of amino acids and UV-absorbing materials into the medium.[58] A bursting of bacterial protoplasts was also observed which could be reversed with equal concentration of phosphatidylethanolamine.

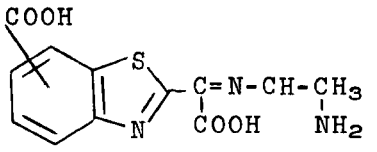
Saramycetin - Saramycetin, known as X-5079C, is a polypeptide antibiotic which possesses limited in vitro antifungal activity.[59] Saramycetin showed only a fungistatic action against Histoplasma capsulatum.[60] The compound was effective against experimental histoplasmosis, partially effective against coccidioidomycosis and blastomycosis, and ineffective against cryptococcosis.[61] Systemic administration is characterized by a low order of toxicity, and there is no oral activity observed.[62] Clinical effectiveness was obtained

in histoplasmosis, North and South American blastomycoses, aspergillosis, sporotrichosis, and phycomycosis.[63] The few side effects reported include eosinophilia and hepatic disfunction; however, a relapse rate of 57% and 33% was reported following treatment of blastomycosis and histoplasmosis respectively.[63]

Saramycetin is characterized by a high sulfur content.[64] Based on the currently proposed molecular weight[65] of 2,100, there are nine sulfur atoms per mole. Two moles each of cysteine, aspartic acid, glycine, threonine, and proline are found on hydrolysis.[66] Two distinct thiazole moieties have been found to date.[66,67] One of these, called saramycetic acid,[66] is 2-(2-acetylthiazol-4-yl)-4-thiazolecarboxylic acid (IV) and is thought to be originally present as the thiazolidine function. The other saramycetoic acid is 2-(3-amino-1-carboxy-2-aza-1-butenyl)-benzothiazole-6 (or 5)-carboxylic acid (V).[67] Compound V readily forms the corresponding imidazolinone ring which was also called saramycetic acid. The thiazole ring system is present in several other antibiotics[66] such as bacitracin A and bottromycin.



IV



V

5-Fluorocytosine - 5-Fluorocytosine is an antimetabolite of cytosine which is inhibitory to a limited number of saprophytic and parasitic fungi.[68] The relatively nontoxic compound is effective against experimental infections of Candida albicans and Cryptococcus neoformans when administered orally or parenterally.[62,69] Clinical efficacy by oral therapy has been reported for systemic candidiasis and cryptococcosis with reversible side effects limited to a mild leukopenia, elevated transaminase levels, and a mild skin rash.[62,70,71] After oral administration of 5-fluorocytosine-2-^{14}C to rats, the known metabolites of 5-fluorouracil: α-fluoro-β-ureidopropionic acid, urea, and CO_2 were observed.[72] In man the compound was recovered unchanged.[72] Cultures of Cryptococcus neoformans collected prior to treatment were inhibited by 0.93-3.9 μg/ml, whereas cultures isolated 2-6 weeks after treatment were resistant to 1,000 μg/ml of 5-fluorocytosine.[73]

Polyenes - The occurrence, production, and sources of polyene macrolide antibiotics have been summarized.[74] The discovery that the antifungal action of polyenes is associated with

their interaction at sterol containing cellular membranes has prompted numerous reports concerning aspects of this phenomenon.[75] Amphotericin B significantly increased the permeability of thiourea across cholesterol, but not phospholipid, containing bilayer membranes.[76] Biologically inactive derivatives of filipin produced a much weaker interaction with cholesterol monomolecular layers than the parent polyene; only a slight interaction was seen when cholesterol acetate replaced the sterol.[77] Interactions between amphotericin B and membrane-bound cholesterol resulted in the formation of aqueous pores with equivalent radii in the range of 7-10.5 A.[78] Filipin, pimaricin, and nystatin produced an insulin-like stimulation of glucose utilization in isolated adipose cells.[79] Amphotericin B was reported to inhibit the uptake of catecholamines by adrenergic neurons in rabbit heart slices and to increase sodium transport and osmotic permeability in amphibian bladders.[80,81]

Amphotericin B - Amphotericin B has been used intravenously for meningitis, pyelonephritis, and pulmonary infections caused by Cryptococcus neoformans, an encapsulated yeast.[82-84] Successful therapy of pulmonary histoplasmosis required 30 mg/kg of amphotericin B while 20 patients with presumed Histoplasma chorioretinitis (eye infection) failed to respond.[85,86] Other reports concern amphotericin B in the treatment of primary renal candidiasis, rhinocerebral phycomycosis, blastomycosis, chromoblastomycosis, geotrichosis, and disseminated infections caused by Torulopsis glabrata and Beauveria bassiana, a fungus normally pathogenic to insects.[87-93] Topical application of amphotericin B was an effective treatment of Candida granuloma and maduromycosis.[94,95]

The nephrotoxicity associated with the use of amphotericin B was found to vary with individual susceptibility, not dose.[96] The adverse effects of amphotericin B on renal function have been summarized.[97] The inhibition of p-aminohippuric acid (PAH) transport in rat kidney by amphotericin B may reflect on the significance of renal blood measurements.[98] Amphotericin B is cleared from cerebrospinal fluid by simple diffusion and not an active transport mechanism.[99] Combination therapy of amphotericin B and sulfonamides resulted in a high frequency of patient intolerance.[100] Close attention to fungal pathogen inhibitory values and peak serum levels permitted less amphotericin B to be used with less toxicity.[101]

Nystatin - An important use of nystatin remains the local treatment of Candida infections; topical applications of this antibiotic are recommended for onychomycosis (nail infections).[102] Additional reports have appeared concerning the use of nystatin or hamycin in combination with tetracycline antibiotics in order to suppress Candida overgrowth which can

occur following tetracycline administration.[103,104] A study with 111 patients with bacterial respiratory infections demonstrated no reduction in the frequency of gastrointestinal side effects in patients receiving tetracyclines with nystatin, although there was a reduction of C. albicans in feces.[105]

Hamycin - Orally administered micronized hamycin was of therapeutic value in blastomycosis[106] but was ineffective against histoplasmosis and cryptococcal meningitis.[107] Mild nausea and anorexia were observed.

Studies on Blastomyces dermatitidis isolated from both improved and unimproved cases receiving hamycin therapy revealed that the variation in clinical response was not due to differences in susceptibility of the organism.[108]

Pimaricin - Pimaricin has been reported to be an effective local treatment for candidiasis, geotrichosis, and otomycosis caused by Aspergillus niger.[109,110] Pimaricin or nystatin administered by inhalation had little effect on bronchopulmonary aspergillosis.[111]

Levorin A - A new polyene of the candicidin group, levorin A, has been described.[112,113] Like trichomycin and hamycin it cannot be separated from candicidin by chromatography.[114]

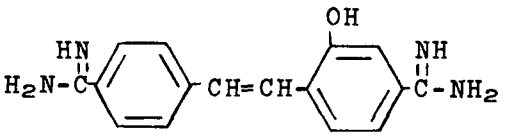
Filipin - Crystalline filipin was isolated in 1955, and the structure determined in 1964. Recently the purity of the crystalline compound was re-examined, and eight chemically related substances were found.[115] Factors II, III, and IV represented 96% of the starting mixture. The remaining minor factors were designated "I complex." A study of the biological properties of these factors indicated that filipin II and III were about equal and much more potent than filipin I or IV, as measured by hemolytic or microbiological activity.[116] Filipin III preferentially disrupted cholesterol liposomes, while filipin II reacted indiscriminately.[116] Mass spectral analysis of derivatives of these factors indicated stereochemical differences as they possessed the same molecular weights.

Hexamycin - A new hexaene antibiotic, hexamycin, has been reported; and its isolation, chemical, and biological properties have been described.[117-119]

Polyene Resistance - Two of 31 in vitro induced nystatin resistant strains of Cryptococcus neoformans showed cross resistance to amphotericin B.[120,121] Studies using three in vitro induced nystatin resistant mutants of Saccharomyces cerevisiae indicated that cross resistance correlated with polyene size.[122] Variants of Candida albicans resistant to

80 units/ml of nystatin were obtained with high frequency; genetic analysis of 12 Saccharomyces mutants revealed that resistance was fully dominant in 8 mutants.[123] Although in vitro acquired resistance to polyenes by yeast is substantiated, the relation of such resistance to the in vivo state remains obscure.

Hydroxystilbamidine - Hydroxystilbamidine (VI) has been reported to be useful clinically against blastomycosis.[124,125] When administered by daily intravenous infusion, an over-all successful treatment rate of 90% with low toxicity was achieved.[126]



VI

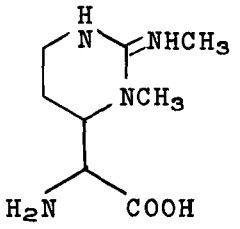
Myxin - The antibacterial and antifungal properties of myxin (6-methoxy-1-phenazinol-5,10-dioxide) have been described.[127] A 1% cream formulation of myxin was an effective treatment of experimental Trichophyton mentagrophytes infections on guinea pigs; however, 3% formulations of several closely related phenazine derivatives were ineffective.[128]

Rutamycin and the Oligomycins - The mechanism of action of rutamycin and the oligomycins allows their use as biological tools in the study of mitochondrial respiration.[129] The molecular weights of the various members of this group appear to be about 850 rather than 450 as had been suggested earlier.[129,130]

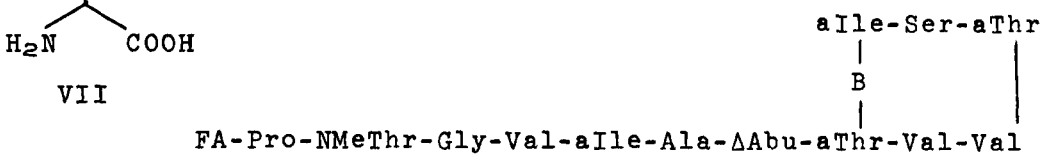
Aflatoxin B - This metabolite, obtained from Aspergillus flavus, inhibited strains of Streptomyces and Nocardia without showing antibacterial activity.[131]

Stendomycin - Stendomycin, a lipid containing polypeptide complex, inhibits a number of fungal plant pathogens.[132,133] Acid hydrolysis showed five components in the fatty acid fraction; the two major being isomyristic and 11-methyl lauric acid.[134] The basic amino acid residue, VII,[135] similar to those of viomycin and capreomycin, (see chapter 9, this section) was found along with dehydrobutyrine[136] and several more common amino acids.[137]

The amino acid sequence and thus the structure of stendomycin was shown to be VIII by classical chemical means as well as mass spectral sequence determination of the permethylation product of stendomycic acid (open form of VIII from mild alkaline hydrolysis).[135,138]



VII

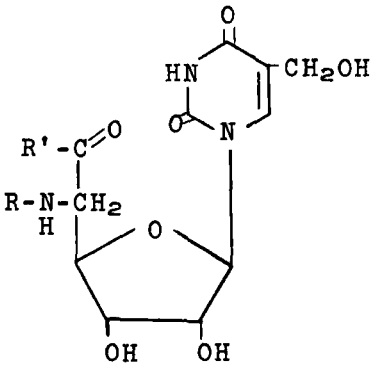


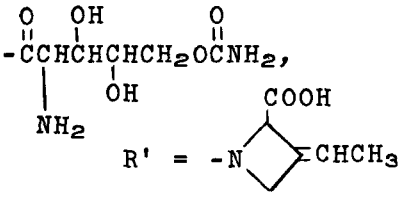
VIII

FA = Fatty Acid
B = VII

Polyoxin - Nine components of the polyoxin complex have been separated and characterized. The structures shown below have been determined for three (A, B, and C) of these glycosidic 5-hydroxymethyluracil (IX) derivatives, which are inhibitory only to phytopathogenic fungi.[139-142] Polyoxins show a chemical relationship to gougerotin (X).[143] The latter substance specifically inhibits protein synthesis by preventing the transfer of aminoacyl-tRNA to protein.[144]

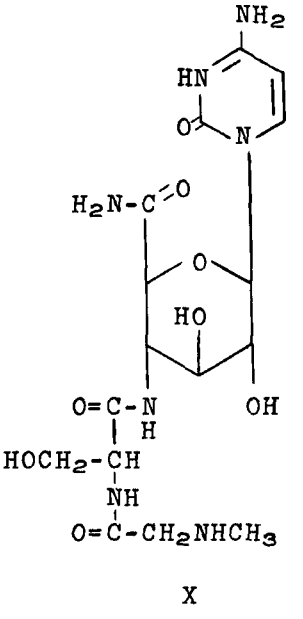
IX



IX-A; R = 

IX-B; R = as in I-A, R' = OH

IX-C; R = H, R' = OH

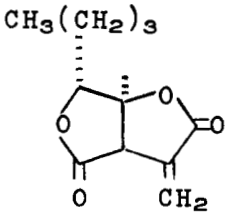


X

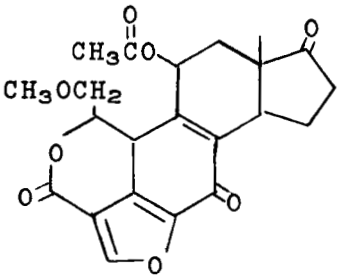
Miscellaneous Antifungal Antibiotics - Two fungal metabolites with in vitro antifungal properties are canadensolide (the dihydroderivative is also naturally occurring) and the

modified steroid, wortmannin.[145-147] Their structures are XI and XII, and they are closely related to avenaciolide and viridin, respectively. Spinamycin, XIII, has been shown to be a succinyl hydrazine.[148,149] Acrylamidine is a streptomycete metabolite with weak anti-Candida activity.[150] There has been recent interest in organic compounds which complex alkali metals. The isolation and characterization of blasticidin A which chelates calcium ion and is inhibitory in vitro to fungi, and selected bacteria has been reported.[151]

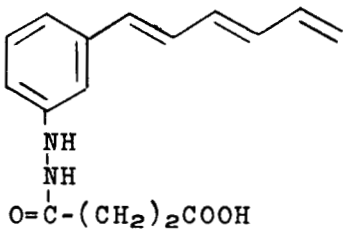
A recent review of the properties of azalomycin F points out that the mechanism of action and general chemical behavior of this compound resembles those of the polyenes.[152] The topical antifungal activity of variotin has been reviewed.[153]



XI



XII



XIII

New Test Methods - A procedure has been established for treating cutaneous Candida albicans infections of the rabbit; triamcinolone acetonide (Kenalog Parenteral®) was used to depress host responses before and after localized infection of the ear lobe.[154] Therapeutic efficacy of topically applied agents was based on a subjective evaluation of the lesions and culture scores.

A 14- to 20-day test system was reported for evaluating the in vivo antifungal activity of compounds against experimental infections of Nocardia asteroides, Histoplasma capsulatum, and Paracoccidioides brasiliensis.[155] Efficacy was based on extension of the mean survival time of untreated as compared with treated mice; the 50% effective dose (ED_{50}) was determined for sulfonamides, amphotericin B, and saramycetin.

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Chapter 14. Antineoplastic Agents

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Introduction - Although agents with greater selective toxicity for cancer cells are badly needed, studies on the kinetics of cell proliferation of L1210 leukemia[1] and the Fortner plasmacytoma[2] in rodents, and the effects of drugs thereon, indicate that by proper scheduling of the administration of presently available agents sufficient selective toxicity can be achieved to consistently effect cures of these experimental diseases.[2,3] The principles established by these and other studies of a similar nature[4] form the basis for significant recent advances in the treatment of human cancer.[5,6] The significance of the cell cycle[7] and the stationary phase (G_0)[8] to drug therapy has only now become apparent,[9-14] and appreciation of the implications of these concepts is reflected in recent clinical work.[15-18] Combination chemotherapy continues to show promise[19,20] both in experimental animal systems[19,20] and in the management of the human disease.[21-28]

Thiosemicarbazones - The observation of the antileukemic activity of 2-pyridinecarboxaldehyde thiosemicarbazone[29] has been extended to the related heterocyclic thiosemicarbazones, 3- and 5-hydroxy-2-pyridinecarboxaldehyde thiosemicarbazones (3-HTS and 5-HTS)[30] and 1-isoquinolinecarboxaldehyde thiosemicarbazone (ITS).[31] Structural modification of ITS has resulted in compounds with greater therapeutic indices than the parent.[32] Biochemical studies have indicated a positive correlation between tumor-inhibitory potency of 3- and 5-HTS and their inhibition of the synthesis of DNA.[33] Further studies have shown that this effect is probably due to the inhibition of ribonucleotide reductase.[34] In addition to these compounds a number of bis(thiosemicarbazones) have shown the ability to inhibit animal neoplasms. Among these are the bis(thiosemicarbazones) (PTS and KTS) of pyruvaldehyde and 3-ethoxy-2-oxobutyraldehyde (kethoxal).[35] The effects of copper and other metals on the activity of PTS and KTS have been studied,[36,37] and it has been proposed that the copper chelate of KTS is the active drug.[38,39] The bis(thiosemicarbazones) are quadridentate ligands[40] that release copper intracellularly, which in turn prevents the incorporation of thymidine into DNA, presumably by interfering with thymidine kinase.[38] Synergism is seen with KTS and 6-thioguanine or N-isopropyl-α-(2-methylhydrazino)-p-toluamide,[41] and the activity of PTS has been shown to be hormone dependent.[42]

Phthalanilides and Related Polybasic Compounds - Studies with 2-chloro-4',4''-bis(2-imidazolin-2-yl)terephthalanilide, one of a series of compounds highly active against leukemia L1210, was shown to completely

inhibit DNA and lipid syntheses at therapeutic levels, but not RNA and protein syntheses.[43] Despite its marked activity against leukemia L1210, 2-amino-4',4''-bis(4-methyl-2-imidazolin-2-yl)terephthalanilide was found to be inactive in clinical trials,[44] as has been the case with other phthalanilides. Cain has studied structure-activity relationships in a series of bisquaternary salts related to the phthalanilides and proposed a mechanism for the action of these structures.[45] Two other similar compounds, 4,4'-diacetyldiphenylurea bis(guanylhydrazone) dihydrochloride[46,47] and 2,2'-(9,10-anthrylenedimethylene)bis[2-thiopseudourea]dihydrochloride,[48] that are active in a number of experimental animal tumor systems may also be similar to the phthalanilides in their action.

Folic Acid Antagonists - Interest in antimetabolites that interfere with the synthesis of nucleic acids continues. Studies on the transport and uptake of methotrexate[49,50] and 2,4-diamino-5-(3,4-dichlorophenyl)-6-methylpyrimidine[50] indicate that clinical response is related to cellular uptake of drug,[49] and the resistance of certain cells to methotrexate appears to be due to lack of transport into these cells.[51] Work on other resistant cell lines indicates that resistance can also be due to an increase in cellular content of folate reductase,[52] but no correlation was observed between resistance and the level of other enzymes involved in folate metabolism.[53] Despite these results there is evidence that the ability of methotrexate to kill cells cannot be entirely explained by its inhibition of folate reductase.[54] Leucovorin at appropriately timed intervals improved the therapeutic index of methotrexate in the treatment of head and neck cancer,[55] and lymphosarcoma and reticulum cell sarcoma.[56] The use of methotrexate in the treatment of hormone-refractory metastatic breast carcinoma[57] and the use of intrathecal methotrexate[58] also appear promising. Oxidation of methotrexate to 7-hydroxymethotrexate by liver aldehyde oxidase[59] is probably a detoxification mechanism. Material previously reported to be tetrahydromethotrexate has now been found to be a mixture of di- and tetrahydromethotrexate, both of which are less effective than methotrexate in the inhibition of folate reductase, but more effective in the inhibition of thymidylate synthetase.[60]

Baker has developed an approach to selective cytotoxicity based on active-site-directed irreversible inhibitors that combine reversibly with the target enzyme and then inactivate it by exo-alkylation.[61] Using this approach, he has prepared compounds that in vitro selectively inactivate folate reductase from leukemia L1210, but not the enzyme from mouse liver, spleen, and intestine.[62] In vivo, however, the only compound that was effective in prolonging the life of mice with L1210 leukemia did not show selectivity for the reductase from leukemic cells.[62] Hansch has applied regression analysis to a series of pyrimidine and triazine inhibitors of folate reductase.[63]

Inhibition of thymidylate synthetase by tetrahydrohomofolic acid does not appear to be responsible for its activity against methotrexate-resistant mouse leukemias; it may be acting by preventing the uptake of folate.[64] A review of the relationship of structure to antitumor activity in compounds related to folic acid has appeared.[65]

Purines - The metabolism and mechanism of action of purine analogs has been reviewed.[66] The finding that 6-(methylthio)purine ribonucleotide, the most potent known feedback inhibitor[67] of de novo purine nucleotide biosynthesis,[68] is an anabolite of 6-mercaptopurine[69] (6-MP) may help resolve the confusion concerning the mechanism of action of this important anticancer agent. At the same time comparative clinical pharmacologic studies[70] on 6-MP and 6-(methylthio)purine ribonucleoside (MeMPR) may explain, at least in part, the synergistic effects observed with these two drugs.[71] The periodate oxidation product of MeMPR inhibited the Ehrlich ascites tumor apparently by blocking DNA synthesis.[72] A number of 6-thiopurines have shown marked activity against the Friend virus leukemia.[73] The tumor inhibition by the anomeric 2'-deoxyribonucleosides of thioguanine has been correlated with their incorporation into DNA.[74] A lipid-soluble derivative of 6-MP, 6-(cyclopentylthio)-9-ethylpurine, appears to be more effective than 6-MP against cancers implanted intracerebrally in mice.[75] Cordycepin-1-oxide[76] and N-hydroxyadenosine[77] have shown good activity in experimental animal systems. 9-β-D-Arabinofuranosyladenine (ara-A) and 9-β-D-xylofuranosyladenine (xylo-A) have both shown some anticancer activity, but their usefulness is limited because of their rapid in vivo deamination. Inhibitors of this deamination have been studied[78] and the 2-fluoro derivatives of ara-A and xylo-A, which presumably are resistant to deamination, have been prepared.[79] Clinical evaluation of the 7-deazapurine ribonucleoside antibiotics and of trimethylpurine-6-ylammonium chloride has been disappointing.[80-82]

Pyrimidines - The leukocytes of patients responding to 1-β-D-arabinofuranosylcytosine (ara-C) exhibited reduced DNA and elevated RNA and protein contents, whereas the normal bone marrow cells of these patients exhibited increased DNA, RNA, and protein contents. Despite extensive intracellular conversion of ara-C to nucleotides, only limited incorporation of the compounds into nucleic acids occurred.[83] 2'-Deoxycytidine reverses the effects of ara-C by inhibiting its phosphorylation,[84] an anabolic step that must occur before it can inhibit DNA polymerase and, consequently, cell proliferation.[85] Uridine exerts a sparing action on ara-C,[86] and the effective level of this drug can be prolonged by coadministration of tetrahydrouridine,[87] a potent inhibitor of deoxycytidylate aminohydrolase.[88]

5-Fluoro-2'-deoxyuridine 5'-monophosphate has been considered to be the cytotoxic anabolite of 5-fluorouracil, but the phosphates of 5-fluorouridine are also anabolites and are incorporated into RNA. The importance of this incorporation to the action of 5-fluorouracil is under investigation.[89-91] L cells in logarithmic growth are much more sensitive to 5-fluorouracil than cells in the stationary phase, but the addition of thymidine following exposure to 5-fluorouracil eliminated the differential.[92] 5-Fluorouracil can be safely administered clinically by slow intravenous infusion[93,94] and appears to be useful as a surgical adjuvant.[95,96] A review of the relation of structure of purine and pyrimidine nucleosides to antitumor activity has appeared.[97]

Other Antimetabolites - The suggestion has been made that the cytotoxic effect of azaserine, the glutamine antagonist, may be due to the depletion of adenine nucleotides that act as activators of thymidine and thymidylate kinases.[98] Azotomycin, a derivative of 6-diazo-5-oxy-L-norleucine (DON), has shown activity against a number of solid tumors in man.[99]

Hydroxyurea is known to inhibit DNA synthesis, and experiments in whole animals have indicated that it does so by preventing the reduction of ribonucleotides to 2'-deoxyribonucleotides, leading to studies of its inhibition of ribonucleotide reductase from a number of sources.[100,101] Still the failure of deoxyribonucleosides to prevent the hydroxyurea effect reveals that additional sites of action may be important,[102] and other work suggests that the lethal effects are a manifestation of a hydroxyurea disturbance in the structure of DNA.[103] Hydroxyurea has some effect against nonresectable cancer of the lung,[104] renal cell carcinoma,[105] and, with radiation, against cancer of the cervix.[106] The activity of guanazole against leukemia L1210[107] also appears to result from its inhibition of ribonucleotide reductase.[108]

Two excellent reviews of L-asparaginase and its antitumor activity have appeared.[109,110] A high remission rate in acute lymphoblastic leukemias in humans treated with L-asparaginase prepared from E. coli has been observed.[111] The L-asparagine analog, 5-diazo-4-oxo-L-norvaline, specifically inactivates L-asparaginase and inhibits the growth of L-asparagine-dependent or L-asparaginase-sensitive tumor cells in culture.[112]

Inhibitors of RNA Synthesis - An excellent review of the antibiotics that are specific inhibitors of DNA-controlled synthesis of RNA, a number of which are excellent anticancer agents (e.g. actinomycin, chromomycin A_3 and daunomycin), has appeared.[113] The structure of mithramycin has been established,[114] and the total synthesis of actinomycins C1, C2, 1-C2, and C3 has been accomplished.[115] One group of investigators

claim that daunomycin is a more effective inhibitor of DNA than RNA synthesis.[116] Others find no difference in its ability to inhibit these two processes, but do not agree on the sensitive phase of the cell cycle.[117,118] One group finds cells in S phase most sensitive,[117] while the other believes the drug interferes during the G2 period.[118] Uptake and retention of daunomycin by leukemic cells are factors in response to the drug.[119] Excellent response of acute granulocytic leukemia to rubidomycin, which has been found to be identical to daunomycin, has been obtained.[120]

Chemically Reactive Compounds - Encouraging reports on the clinical activity of cyclophosphamide,[121-123] other analogs of nitrogen mustard,[124-128] and mitomycin[129] continue to appear. Comparative studies of cyclophosphamide[130] and nitrogen mustard[131] with the vinca alkaloids in the management of Hodgkin's disease showed little difference in the effectiveness of these two classes of agents. Some clinical activity has been observed with a new methanesulfonate, piposulfan.[132,133] Potentiation of the therapeutic effect of radiation with AB-132 in thoracic malignancies has been reported.[134]

Unequivocal, dramatic, and rapid responses were obtained with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in 17 of 31 patients with advanced Hodgkin's disease resistant to the standard chemotherapeutic agents,[135] but this drug was only minimally effective in advanced gastrointestinal cancer.[136] Pharmacologic studies of another nitrosourea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), in animals have been described.[137] 5(or 4)-(3,3-Dimethyltriazeno)imidazole-4(or 5)-carboxamide has shown promise in the treatment of a number of solid tumors and in acute myelogenous leukemias, but it has been used most successfully in the treatment of malignant melanoma, some cases of which respond dramatically.[138] The effects of nitrosoureas and alkylating agents on leukemia L1210 resistant to 5(or 4)-[3,3-bis(2-chloroethyl)triazeno]imidazole-4(or 5)-carboxamide has been studied.[139]

Miscellaneous Agents - The papers from a symposium on vincristine, which also covers the other vinca alkaloids, have been published.[140] Vincristine has shown activity against a variety of cancers in children and is considered to be a very useful clinical drug.[140,141] The mechanism by which these alkaloids inhibit tumor growth remains unknown. Although they appear to produce metaphase arrest, this effect does not correlate with therapeutic response.[140,142] The ability of the vinca alkaloids and a number of other plant products to function, with imidazole catalysis, as biological acylating agents is the basis of a unifying hypothesis that has been presented to explain the activity of these compounds.[143] A review of the entire clinical history of streptonigrin shows it to be a compound with a low therapeutic index and a limited range of

effectiveness. It seems to have some value in the treatment of Hodgkin's disease, lymphosarcoma, and mycosis fungoides.[144] The marked effects of streptonigrin on cellular metabolism has been attributed to its catalytic oxidation of NADH and the resultant peroxide formation.[145]

Structure-activity studies of the acetylenic carbamates continue.[146] The activity of lapachol,[147] 1-(4-dimethylaminobenzylidene)-indene,[148] poricin,[149] and mycophenolic acid[150] against experimental animal tumors was reported. Metastatic lung cancer was found to respond to treatment with hexamethylmelamine.[151] An experimental interferon-inducing agent—polyinosinic:polycytidylic acid, which is used against virus infections, has now been found to inhibit tumor growth in experimental animals. This antitumor activity is not believed to depend on the antiviral action of interferon, however.[152]

Immunosuppressive Agents - Although progress made in the past several years in serologic matching of graft donors and recipients has greatly reduced the immunologic gap, that which remains will probably have to be bridged with immunosuppressive agents.[153] So far the drugs used for this purpose—methotrexate, cyclophosphamide, 6-mercaptopurine, azathioprine, 5-fluorouracil, etc.—have been drawn from the cancer program. Unfortunately, the incidence of reticulum cell sarcomas and related tumors in patients who have received organ transplants and have been given immunosuppressive therapy is high enough to cause concern,[154,155] since it is known that most, if not all, anticancer agents are also carcinogenic under certain conditions. Two excellent reviews of chemical suppression of the immune response have appeared.[156,157]

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Section IV - Metabolic Diseases and Endocrine Function

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Chapter 15. Antidiabetics

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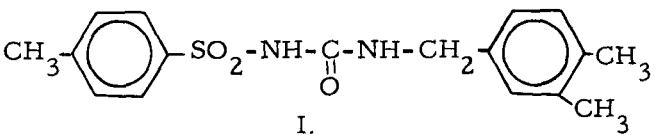
In last year's chapter the current status of basic research in diabetes mellitus was reviewed and evaluated. The reason for taking that approach was that a great .eal more knowledge must be obtained about the biochemical aspects of diabetes before the disease can be prevented or satisfactorily treated. This reviewer feels that the relative advancements in most of the basic aspects of diabetes research are comparatively small during the course of a given year, so he chooses not to make this year's chapter simply a continuation of last year's. Instead, the present chapter is devoted to a discussion of the study of new oral hypoglycemic agents.

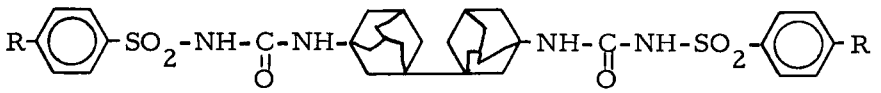
Sulfonylureas- Four sulfonylurea hypoglycemics are currently on the American market. A great deal of information has been published on the actions and uses of these compounds. Much of this information has been summarized in a recent symposium. [1] The results of clinical studies with the sulfonylureas continue to be published[2-7] but little new information has been presented on their mechanisms of action. It is still generally felt that they act by stimulating pancreatic insulin release but the mechanism by which they accomplish this is poorly understood.

Several studies on the synthesis and evaluation of new sulfonylurea hypoglycemic agents have been reported during the year. One such study involved the synthesis and testing of a series of N-benzyl-N'-p-toluenesulfonylureas related to I. Many of these compounds were found to be effective hypoglycemics in rats. [8] Compound I was found to be the most active member of the series. It is comparable to tolbutamide in potency.



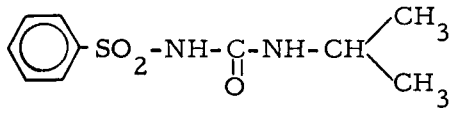
I.

The synthesis and evaluation of a series of 1, 1'-biadamantylarylsulfonylureas has been reported. [9] The general structure of these compounds is illustrated by II.



II.

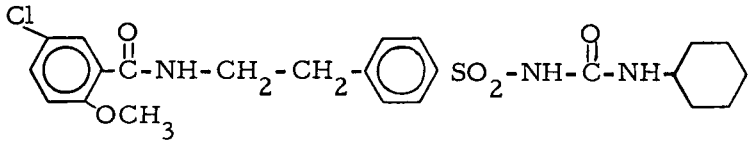
For the most part, these compounds were found to be comparable in potency to other N-adamantyl-N'-arylsulfonylureas which had been studied earlier. [10] The new compounds exhibit no important advantages over the earlier compounds and for that matter, they possess the disadvantage of having a delayed onset of action. [9] The total duration of action of the biadamantane derivatives is not significantly different from that of the earlier adamantane derivatives. [9]



III.

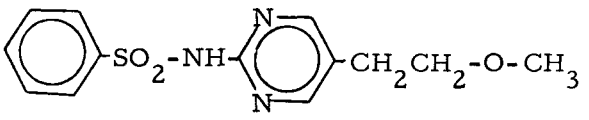
N-Benzensulfonyl-N'-isopropylurea (III) was found to be the most active member of a series of sulfonylureas synthesized and tested by another group of investigators but this compound has no particular advantages over existing compounds. [11]

Studies with newly synthesized acylaminoalkylbenzenesulfonylureas have produced some extremely potent oral hypoglycemics. [12] It is somewhat surprising to find such a high order of potency in compounds of this type inasmuch as N-(4-acetamidobenzenesulfonyl)-N'-n-butylurea, a metabolite of carbutamide, is devoid of hypoglycemic activity. This emphasizes the importance of the alkyl chain which is interposed between the benzene ring and the amide function. The most active compound to result from this study is HB 419 (IV) which is capable of lowering blood sugar in humans at doses of less than 0.1 mg/kg. [12] Like the other sulfonylureas, this compound is not active in pancreatectomized animals or in a variety of isolated organ systems. [12] Accordingly, it seems quite probable that it has the same mechanism of action as other sulfonylurea hypoglycemics. The acute toxicity of HB 419 in mice is quite low and may be even lower than that of tolbutamide. [12]



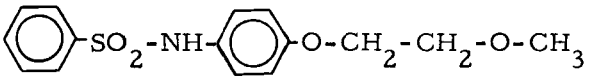
IV.

Further studies on the hypoglycemic activity of glycodiazine (V) have been reported. [13, 14] This compound is not a sulfonylurea but it is structurally related to the sulfonylureas and it apparently exerts its action by stimulating pancreatic insulin release. [15] Recent studies indicate that glycodiazine is a potentially useful oral hypoglycemic for the treatment of adult-onset diabetes. [13]



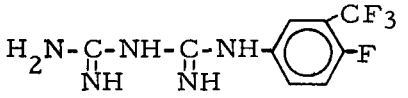
V.

Glymidine (VI), another compound which is structurally similar to the sulfonylureas, has been subjected to clinical trials in humans. [15] Oral administration of this compound causes a rise in plasma levels of immunoreactive insulin[16] which probably accounts for its ability to function as a hypoglycemic agent. It is a potentially useful compound with a potency which is intermediate between tolbutamide and chlorpropamide. [15]



VI.

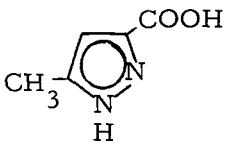
Biguanides- A great deal of research has been conducted on the metabolic effects of phenformin and other biguanides. The current status of phenformin in the treatment of diabetes was the subject of a recent symposium. [17] The symposium dealt with the absorption, distribution and excretion of phenformin as well as with its effects on glucose tolerance, plasma insulin levels, serum lipids, body weight, and lacticacidemia. The original suggestion of Steiner and Williams[18] that phenformin exerts its action on glucose metabolism by inhibiting certain aerobic processes seems quite reasonable in light of current knowledge of its metabolic effects. Certain objections to this hypothesis have been voiced but it is generally believed that phenformin acts by blocking energy transfer at the cytochrome b site of the electron transport chain. [19] The resulting inhibition of oxidative phosphorylation could then account for the metabolic effects of phenformin (inhibition of pyruvate and citrate oxidation, inhibition of hepatic gluconeogenesis, increased lactate and pyruvate levels, and decreased serum triglyceride and cholesterol levels). [19] The use of biguanides in combination with sulfonylureas has certain advantages and is becoming more popular in clinical practice. [20] The metabolic effects of administering a combination of tolbutamide and buformin to maturity-onset diabetics have been studied. [21, 22] Good control of blood sugar levels can be obtained in the presence of lower plasma insulin levels than is the case when a sulfonylurea is employed alone. [21] A series of trifluoromethylphenylbiguanides was prepared and tested for hypoglycemic activity in guinea pigs. [23] One member of the series (VII) was found to be 10 times more active than the reference compound,



VII.

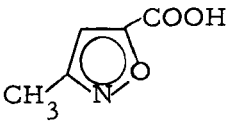
N,N'-dimethylbiguanide. [23] All of the compounds except one exhibited anorexic activity. [23]

Pyrazoles and Isoxazoles- Further in vitro[24] and in vivo[25,26] studies have been conducted with 5-methylpyrazole-3-carboxylic acid (VIII). This compound is known to depress blood sugar levels in fasted, glucose-primed rats by a mechanism which may be secondary to its depression of plasma free fatty acid (FFA) levels. [27] In vitro studies have demonstrated that it inhibits lipolysis, increases glycogen synthetase activity, and inhibits its phosphorylase. [24] The inhibition of lipolysis is probably responsible for its effect on plasma FFA levels. Although it inhibits basal, epinephrine-induced and theophylline-induced lipolysis in vitro, it does not inhibit lipolysis stimulated by dibutyryl cyclic AMP and it has no effect on the hydrolysis of cyclic AMP by phosphodiesterase. [28] Accordingly, it is quite conceivable that this compound exerts its action by inhibiting adenyl cyclase. 5-Methylpyrazole-3-carboxylic acid stimulates glucose metabolism in adipose tissue more markedly in vivo than in vitro but it has no effect on glucose metabolism in the diaphragm. [25] This drug does not depress blood sugar in normal rats but it does prevent the rise in blood sugar which is normally induced by mannoheptulose or by anti-insulin serum.[25] Studies on the effects of this pyrazole in man have revealed that it lowers plasma FFA levels and inhibits the adipokinetic effects of epinephrine and growth hormone but it does not alter blood sugar levels. [29] The effect of this agent on FFA levels in rats is diminished and abbreviated after 4 days pretreatment with the drug. [30] Furthermore, this agent does not lower blood sugar levels significantly in rats after 4 days pretreatment. [30] The abbreviated depression of FFA levels induced in the pretreated animals may not be of sufficient length or magnitude to produce a fall in blood sugar. Resistance to this agent is apparently related to adrenal and/or hypophyseal function since the removal of these glands abolishes tachyphylaxis whereas the adrenalectomized, hydrocortisone-treated rat does not develop tachyphylaxis. [30] Resistance to this pyrazole is apparently not related to an alteration in its metabolism since its metabolism is the same in both pretreated and nonpretreated rats. [31]

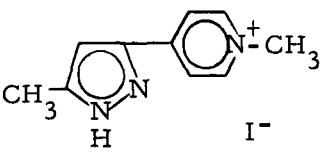


VIII.

Resistance to 3-methylisoxazole-5-carboxylic acid (IX) has also been demonstrated. [32] This compound is similar to the corresponding pyrazole in that it depresses plasma FFA and glucose levels in fasted glucose-primed rats[33], and the effect is lost after 2 days pretreatment. [32] Resistance to the isoxazole is also dependent on an intact pituitary-adrenal system. [32]



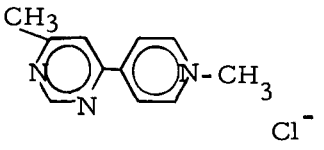
IX.



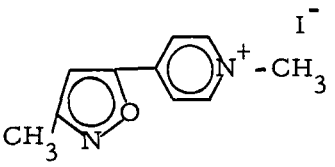
X.

Recent studies have demonstrated that oral administration of 1-methyl-4-(5-methyl-3-pyrazolyl)pyridinium iodide (X) lowers blood sugar levels in fasted normal chicks and in alloxan-diabetic mice.[33]

Several analogs of this compound have been synthesized and tested and it is apparent that the substituted pyrazole ring must be attached in the 4-position of the pyridine ring.[33] The pyridinium salt moeity is an important factor inasmuch as the analogous tertiary base lacks hypoglycemic activity.[33] The 5-methyl group can be replaced by a number of other groups without loss of activity but activity is destroyed when certain electronegative substituents are placed in this position.[33] Attempts to replace the pyrazole ring with a pyrimidine ring have been unsuccessful.[34] 1-Methyl-4-(4-methyl-6-pyrimidyl)pyridinium chloride (XI) and several of its analogs failed to significantly lower blood sugar in mice or chicks.[34]



XI.



XII.

It is not surprising that hypoglycemic activity is retained when an isoxazole ring is substituted for the pyrazole ring.[35] 1-Methyl-4-(3-methyl-5-isoxazolyl)pyridinium chloride (XII) and several of its analogs possess hypoglycemic activity comparable to the analogous pyrazole derivatives.[35,36] This compound is also effective in alloxan-diabetic animals.[35]

Although no detailed studies on the mechanism of action of these newer pyrazoles and isoxazoles have been reported, it seems quite likely that they act by the same mechanism as do the earlier compounds (5-methylpyrazole-3-carboxylic acid and 3-methylisoxazole-5-carboxylic acid) and they are probably subject to the same limitations as the previously studied compounds.

A series of 1-(2, 4-dinitrophenyl)-3, 5-dimethyl-4-arylazopyrazoles[37]

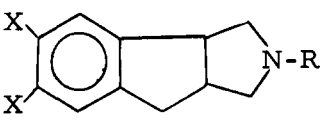
and a series of 1-(2-4-dinitrophenyl)-3-methyl-4-arylazo-2-pyrazolin-5-ones[38] have been synthesized as potential antidiabetic agents. Certain of these compounds have shown some hypoglycemic activity in guinea pigs.

Pyrazinamide- Pyrazinamide (XIII), a drug which has been used clinically as an antitubercular agent, is now being studied for its hypoglycemic properties.[39]

XIII.

It is effective in lowering blood sugar and FFA levels in intact, adrenalectomized and hypophysectomized rats.[39] This compound is metabolized to 2-carboxypyrazine, which is responsible for its action.[39] This is substantiated by the finding that pyrazinamide does not increase glucose oxidation or inhibit FFA release from adipose tissue in vitro whereas 2-carboxypyrazine exerts both actions.[39] Hypoglycemic activity is attributed to the ability of 2-carboxypyrazine to increase glucose utilization in adipose tissue.[39]

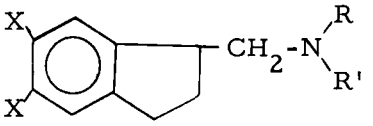
Indans- A series of indanopyroles (XIV) has been synthesized and tested for hypoglycemic activity.[40] Some of these compounds were found to be comparable to tolbutamide in potency when tested in normal rabbits.[40] They were found to be active in moderately alloxan-diabetic animals but not in severely diabetic animals.[40] The relative potency of the various compounds is quite variable and is dependent on the nature of the R group. Compounds with X=OCH_3 are more potent than those with X=H.[40]



XIV.

X= H or OCH_3

R= alkyl, cycloalkyl or benzyl



XV.

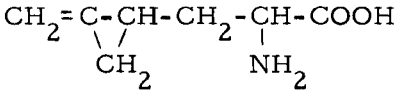
X = H or OCH_3

R and R' = alkyl or H

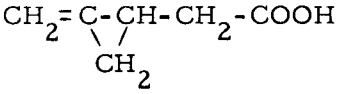
The same workers have also reported on a series of 1-dialkylaminomethylindans.[40,41] These compounds (XV) might be considered to be open-chain analogs of the indanopyroles. Some of the dialkylaminomethylindans exhibit hypoglycemic activity in rabbits but none are as active as the corresponding indanopyroles.[40] Activity is greatly influenced by the nature of R and R'.[40] The more potent dialkylaminomethylindans are those without methoxy groups in positions 5 and 6.[40]

Unsaturated Fatty Acids- Hypoglycin (XVI), a short chain unsaturated amino acid derived from the fruit of Blighia sapida, causes hypoglycemia in

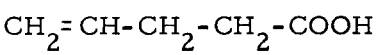
man and in laboratory animals. [42] The biological effects of this compound cannot be duplicated in in vitro systems, thus suggesting that the compound may be converted to an active metabolite in the body. It has recently been shown that it is converted to an α-ketoacid by transamination and then to methylenecyclopropylacetic acid (XVII) which is the active form of the drug. [43] The biological effects of methylenecyclopropylacetic acid and of a similar compound, 4-pentenoic acid (XVIII), have been studied in vivo[44,45] and in vitro.[46] Some of the biological effects of these compounds are: (1) a decreased rate of long chain fatty acid oxidation, (2) a decreased rate of gluconeogenesis, (3) decreased levels of free carnitine, and (4) increased levels of acylcarnitines. [45] Hypoglycin-induced hypoglycemia and depression of palmitate oxidation in mice can be blocked by the administration of carnitine to the animals. [47] The aforementioned statements led to the postulation that these compounds may exert their effect by forming nonoxidizable acyl derivative of coenzyme A. [45] This would decrease the amount of available free carnitine and would inhibit the formation of acylcarnitines from long chain fatty acids, thus resulting in a decreased rate of fatty acid oxidation. [45] It is quite conceivable that the decreased fatty acid oxidation is responsible for the decreased rate of gluconeogenesis[48] and also for the shift to the utilization of glucose as the major energy source in these animals. [45] It should be pointed out that hypoglycin and similar compounds exhibit a rather high degree of toxicity, a factor which must be overcome before any clinically useful hypoglycemics of this type can be produced.



XVI.



XVII.



XVIII.

Salicylates- It has been known for nearly a hundred years that salicylates can lower blood sugar levels in humans and that this effect is more pronounced in diabetics than in normal individuals. [49] Their clinical usefulness is limited by the high dose which is required to maintain euglycemia in diabetic subjects and by the side effects which occur at these dosage levels. The situation is further complicated by the fact that at high doses these compounds may cause hyperglycemia. [50] It has been generally believed that the hypoglycemic effect of the salicylates is related to their ability to uncouple oxidative phosphorylation and thereby increase glucose

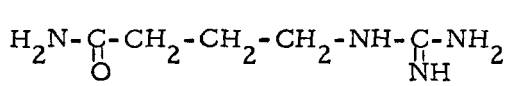
utilization.[51] The mechanism by which these compounds act as uncoupling agents has recently been studied and it was found that it is the free salicylates rather than their acetylated derivatives which are responsible for the uncoupling effect.[52] It should be pointed out that the salicylates have other effects which may contribute to their hypoglycemic activity. They depress plasma FFA in alloxan-diabetic rats prior to their effect on blood glucose levels.[53] It was suggested that the mechanism of hypoglycemic action of the salicylates may be related to a primary effect on plasma FFA levels and the reciprocal relationship between glucose utilization and plasma FFA levels.[53] Others, however, have shown that salicylate infusion in humans can cause a substantial decrease in blood glucose levels without having a significant effect on plasma FFA levels[54] so it is unlikely that this accounts entirely for the hypoglycemic action of the salicylates. There has been some controversy as to whether or not salicylates increase plasma insulin levels by stimulating insulin release from the pancreas.[54,55] A recent study has shown that salicylate infusion into healthy humans and patients with mild diabetes causes a significant increase in immunoassayable plasma insulin levels.[54] This suggests that the mechanism of hypoglycemic action of salicylates may be at least partially related to their ability to stimulate insulin release. The observation that salicylates lower blood sugar levels in alloxan-diabetic rats[53] does not preclude this possibility since these animals are not totally devoid of insulin.

The search for a clinically useful salicylate hypoglycemic continues. In a recent clinical study, 10 maturity-onset diabetics were controlled with 2,3-cresotic acid (2-hydroxy-3-methylbenzoic acid) or a combination of this compound and insulin. 2,3-Cresotic acid was well tolerated in all cases. The authors feel that the compound may act by stimulating insulin release from the pancreas.[56] The relationship between chemical structure and hypoglycemic activity in salicylates and salicylate analogs has been studied.[53] It was found that the carboxyl group of salicylic acid is important to its hypoglycemic activity. The o-hydroxyl group is beneficial but not essential for hypoglycemic activity inasmuch as it can be replaced by other groups without loss of activity.[53] Alkylation of the benzene ring decreases potency and increases toxicity to an extent proportional to the number of carbons in the side chain.[53]

Tris- Tris [tris-(hydroxymethyl)aminomethane] has hypoglycemic activity when infused into animals. The mechanism by which it exerts this effect has been unclear. It stimulates glucose utilization by skeletal muscle in dogs[57] and it is more effective in normal than in pancreatectomized dogs.[58] This leads to the speculation that its mechanism of action may be the stimulation of insulin release from pancreatic beta cells. An inability to detect a significant change in plasma insulin levels following the infusion of tris did not support this hypothesis.[59] These studies, however, used the rat hemidiaphragm assay to measure plasma insulin levels. More recently, it

has been shown that tris infusion does increase immunoassayable insulin levels in the portal vein of dogs.[60] The increase in plasma insulin levels precedes the hypoglycemic effect.[60] In this light, it seems quite likely that the hypoglycemic effects of tris is the result of its ability to promote insulin release. Both the hypoglycemic effect and the insulin releasing effect of tris are greater when it is infused at pH 10.2 rather than pH 7.4.[60] This may be due to the decreased ionization of the base at the higher pH which facilitates the entry of the drug into the pancreatic beta cells.

γ-Guanidinobutyramide- γ-Guanidinobutyramide (XIX), a compound with structural similarities to arginine and phenformin, has been found to lower blood sugar and urea levels in diabetic humans and in laboratory animals.[61] This compound does not stimulate insulin secretion as do the sulfonylureas but it does increase the sensitivity of peripheral tissue to insulin and it stimulates peripheral glucose uptake.[61] It is relatively non-toxic (LD_{50} in mice = 1.725 gr/kg) and it is hypoglycemic in all species tested.[61] Too little is known about the biological effects of this compound to establish its mechanism of action but its hypoglycemic activity may be related to its ability to lower blood urea levels and to influence amino acid metabolism.[61]



XIX.

Ouabain- Ouabain, a cardiotonic glycoside from Strophanthus gratus, lowers blood sugar when infused into dogs.[62] It is also active in a variety of in vitro systems. This glycoside stimulates glucose uptake, glucose oxidation, and total lipid synthesis from glucose in adipose tissue cells.[63] It also inhibits spontaneous[64] and epinephrine-induced glycogenolysis and lipolysis.[65, 66] These effects may be mediated by changes in cyclic AMP levels inasmuch as ouabain has been shown to inhibit adenyl cyclase activity and decrease cyclic AMP production.[66] These results suggest a close relationship between ouabain's effect on glucose metabolism and its effect on lipolysis but they do not establish the actual mechanism by which it lowers blood sugar levels in intact animals. The fact that ouabain is unable to lower blood sugar levels in pancreatectomized animals makes it seem unlikely that its hypoglycemic effect is a result of these actions on tissue metabolism.[67] Recent studies have demonstrated that ouabain increases pancreatic insulin release.[68] This is in agreement with earlier studies which had shown that it stimulates insulin secretion by pancreatic tissue in vitro.[69] The fact that its hypoglycemic effect in dogs occurs subsequent to its effect on plasma insulin levels makes it seem quite likely that its ability to lower blood sugar is a result of its ability to promote insulin release. The possibility of ouabain ever becoming a clinically useful hypoglycemic is quite remote since it cannot be administered orally and since it has a diversity of pharmacological actions.

Monoamineoxidase Inhibitors- It has been observed that certain hydrazine monoamineoxidase inhibitors potentiate the hypoglycemic effect of insulin and sulfonylureas in diabetic patients being treated for depression. [70] This effect has been noted with pargyline, mebanazine, pheniprazine, phenelzine, and other similar drugs. [71,72] The hypoglycemic effect of mebanazine and phenelzine in diabetics who were not suffering from a depressive illness has recently been studied and it was found that these compounds are effective in lowering blood sugar and improving glucose tolerance in non-depressed patients. [73] The mechanism by which these compounds lower blood sugar is poorly understood and a great deal more information must be obtained before a valid hypothesis can be made.

Recent studies have demonstrated that the intraperitoneal administration of tranylcypromine, a nonhydrazine monoamineoxidase inhibitor, produces a slight initial rise in plasma glucose levels which is followed by a profound hypoglycemia. [74] It is proposed that the initial rise in blood sugar is due to a stimulation of hepatic glycogenolysis. [74] The hypoglycemic activity of the compound may be related to its ability to promote insulin release from the pancreatic beta cells. [74] Its ability to stimulate insulin release does not seem to be related to its effect as an MAO inhibitor since other MAO inhibitors (pargiline, nialamid, and marsalid) failed to promote insulin release in this study. [74] Its action is more likely related to its effect on the sympathetic nervous system since its action on insulin release is inhibited by the prior administration of a beta-adrenergic blocking agent and stimulated by the administration of an alpha-adrenergic blocking agent. [74] This is consistent with the fact that insulin release is promoted by the stimulation of pancreatic beta-adrenergic receptors with isoproterenol [75] and inhibited by the stimulation of pancreatic alpha-receptors with norepinephrine. [76] It is also known that the stimulation of beta-receptors in isolated pancreatic islets produces increased levels of 3', 5'-cyclic AMP. [77] The increased cyclic AMP levels probably result from a stimulation of adenyl cyclase which has been proposed to be the beta-adrenergic receptor. [78] The finding that insulin release in the isolated perfused pancreas is also stimulated by the addition of exogenous cyclic AMP is further indication that beta-adrenergic stimulation of insulin release is mediated by this nucleotide. [79] Accordingly, it seems likely that the hypoglycemic activity of tranylcypromine may be attributed to its ability to stimulate pancreatic beta-adrenergic receptors which increases intracellular levels of cyclic AMP and promotes the release of insulin.

The wide diversity of pharmacological actions attributed to the MAO inhibitors and toxicity [80] may preclude their acceptance as clinically useful hypoglycemic agents but it is possible that studies with other compounds of this type could produce new compounds which are effective hypoglycemic agents but do not possess the disadvantages of the present ones.

Miscellaneous Hypoglycemics- Hypoglycemic activity has been reported in a variety of other compounds. N, N-Diethyl-2-[2-(1-hexahydroazepinyl) ethyl]-1-piperidine carboxamide was found to lower blood sugar in fasted glucose-primed rats.[81] Beta-glycyrrhetinic acid and a group of structurally related compounds were found to significantly lower blood sugar when administered orally to rats.[82] Guanethidine, an antihypertensive agent, lowers blood sugar and improves glucose tolerance in some diabetics being treated for hypertension.[83] Hypoglycemic activity has been discovered in a series of polycarbonyl compounds derived from benzoquinone.[84] Studies on the mechanism of action of these quinones suggest that they may act by stimulating insulin release.[85]

It is quite evident from the above statements that the ideal antidiabetic drug has yet to be discovered. Many of the newer drugs show some promise as potential oral hypoglycemics but none of them appear to have any major advantages over the hypoglycemic agents which are currently in use. Many of the newer agents act by stimulating the release of pancreatic insulin. Compounds of this type are dependent on the presence of a functioning pancreas and they cannot be used to treat all diabetics. Other compounds are too toxic to ever become clinically useful. Still others have too great a diversity of pharmacological actions to ever be practical as antidiabetic drugs. The pyrazoles and the isoxazoles held a great deal of promise for some time but certain disadvantages of these compounds have been revealed and it is unlikely that they will become clinically useful hypoglycemics. It is possible that one of the compounds discussed in this review will someday serve as a model for compounds which will possess all of the qualities which are desired in an antidiabetic drug. It seems more reasonable, however, to assume that more information about the basic biochemical abnormalites of diabetes will be needed before a completely effective treatment can be developed.

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Chapter 16. Atherosclerosis

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Introduction - Atherosclerosis, defined at autopsy, can be correlated with both hereditary and environmental factors. No single factor has been found exclusive in the etiology of atherosclerosis in any part of the arterial system. Recognition of one's hereditary risk factors can provide a basis of prophylactic approach to forestall or even circumvent the disease. On the other hand, in contrast to the limited possibility of modifying the hereditary factors, some degree of control can be exerted over the environmental factors. Identification of these factors as relevant to the disease, atherosclerosis, is not simple and often is based on epidemiological studies and statistical evaluation of the data. The possibility of predicting coronary heart disease risk depends not only on the nature of each factor, but also on the interaction of multiple factors and the degree of severity and duration of each factor, singly, and among the others. The most prominent environmental factors in atherosclerosis are elevated serum cholesterol, elevated blood pressure, diet, and smoking. Age is a factor all by itself. Conditions such as concurrent or predisposing disease, lack of exercise, obesity, elevated serum triglycerides, hormone imbalance, etc., are examples of additional factors to consider. In spite of what may appear as almost unconquerable obstacles, progress has been made in pointing out some of the more obvious and prominent factors even though we have not yet mastered control of the disease.

Etiology - Much has been written on the histopathology of atherosclerosis, particularly the nature of the lesion, its development, and its regression , but, as yet, no definitive proof of the exact etiology or initiating event has been set forth. In human atherosclerosis, general evidence suggests that the earliest arterial lesion is a non-lipid lesion consisting of fragmentation of the internal elastic membrane of the intima permitting penetration of materials not normally associated within the intimal and medial layers. However, in studies of human atherosclerotic aortas, no basis was found "to assume that anomalies of the vasa vasorum are a causative factor in the genesis of atherosclerosis."[1] On the other hand, aortas of rats that had been damaged chemically in utero indicated "that the presence of a postlathritic medial scar accelerates or modifies the evolution of atheromatous lesions."[2] Thus, it seems some condition, still not defined with certainty, that damages the arterial wall can produce a focal point in initiating the atherosclerotic process. But what about the conditions that lead up to the first arterial change and the factors that propagate its development into full blown atherosclerosis?

Smoking is among the four environmental factors that correlate most closely with atherosclerosis identifiable at autopsy. Several studies have shown that cholesterol was higher, with coronary heart disease (CHD) and atherosclerosis more prevalent, among cigarette smokers than among non-smokers.[3,4,5] Nicotine injected into dogs daily (subcutaneously) for

six months caused a reduced "turn-over" of cholesterol with no effect on serum cholesterol level.[6] In rabbits, subcutaneously injected nicotine enhanced the hypercholesterolemic properties of the diet and produced more extensive aortic intimal disease than in controls.[7] Nicotine and Vitamin D together caused atherosclerosis in rabbits.[8] Among 1,363 industrial employees, smokers consistently had significantly higher serum B-lipoprotein and serum cholesterol levels than non-smokers.[9] Hypercholesterolemia interacts synergistically with cigarette smoking to increase the risk of lung cancer in a study of 1,594 men.[10]

Elevated serum cholesterol, a major correlating factor, still receives a prominent share of the effort in atherosclerosis research. Even though cholesterol appears elevated in man in this disease state, its elevation may not be a primary cause but merely a response - normal or abnormal - to a metabolic stimulus. Maybe if this elevated cholesterol in humans is not coupled with other contributory factors, abnormal deposition of lipid and cholesterol would not occur and, therefore, little or no atherosclerosis would result. On the other hand, if other factors are present without serum cholesterol elevation, does the disease occur? (In certain experimental animals, however, serum cholesterol elevation appears atherogenic as a single factor). Regardless of lack of absolute proof of cholesterol's role in causing or assisting some other agent or condition in causing atherosclerosis, control of cholesterol metabolism, serum and tissue levels, absorption, and excretion can do little harm and possibly much good in control of the disease. Hypercholesterolemia is not likely to be the only abnormality present in an individual with the cardiovascular disease, atherosclerosis , and it appears synergistic. Cholesterol research is still needed but with some new approach. In addition to cholesterol, correlation between plasma triglycerides and the severity of coronary atherosclerosis in humans with a variety of disease conditions, as evidenced by selective coronary arteriograms, has been found highly significant.[11]

Another factor that has gained considerable attention in the etiology of atherosclerosis is elevated blood pressure. This factor alone does not appear atherogenic until other "contributory" conditions appear. Hypertensive rats on a high-fat, high cholesterol diet developed hypercholesterolemia and atherosclerosis of the coronary arteries within a 4 month period. The aortas were grossly negative, but some rats had slight lipid deposits in the aortic intima and/or media, as seen microscopically.[12] When a blood pressure lowering agent hydralazine (H) was included in a high-fat diet (no cholesterol added) of unilaterally nephrectomized rats, blood pressures remained normal in the H treated groups but were quite elevated in the operated controls. Serum cholesterol was elevated in all groups by 15 weeks and remained high for 30 weeks when the study terminated. Even with marked hypercholesterolemia, the H treated rats had strikingly fewer, and less severe, lipid lesions and atherosclerosis of the coronary arteries than the untreated controls.[13] Short term "hypertension" did not result in an accelerated cholesterol uptake by female aorta, nor did long-term hypertension produce significant aortic atherosclerosis.[12,13,14] Blood pressure, plasma cholesterol and blood clotting

mechanisms were found casually involved in avian atherosclerosis.[15]

Significant correlations were found in humans between the mean values of systolic and diastolic blood pressures, serum triglycerides, and serum cholesterol over 4 years.[16] In reviewing the relation of hypertension to atherosclerosis of coronary arteries, one author concluded, "The rate of atherogenesis is directly related to the blood pressure (or transmural pressure) in all arteries." "Hypertension promotes atherogenesis in the heart, as well as in the brain, kidney, and other organs."[17] There seems to be little doubt that lowering blood pressure favorably affects the rate of atherogenesis in animals. Among diabetics there is a greater than average prevalence of hypertension. A large number of diabetics die of cardiovascular disease--atherosclerosis of the arteries of the heart, brain and limbs, and lesions of the small arteries of the kidney and retina. "It is a source of frustration to the physician and disappointment to the patient that careful, effective control of the diabetic state apparently has little effect in preventing the complications of vascular disease."[18] This statement does not seem to be true in the case where control of hypertension is achieved. "Persons with hypertension or diabetes mellitus consistently have more atherosclerosis in the coronary arteries and the abdominal aorta than persons without hypertension or diabetes, regardless of sex, age, race, or geographic location.[19] Comparison has been made of coronary and aortic atherosclerosis in autopsied persons having cancer (lung, stomach, etc.), cirrhosis of the liver, and tuberculosis; only lung cancer had a tendency (non-significant) to be associated with more severe atherosclerosis (aortic).[20] Even with some differences, cerebral atherosclerosis parallels the various other manifestations of the general disease in coronary atherosclerosis.[21,22] Even though the risk of athero-thrombotic brain infarction may be great in hyperlipemic, hypercholesterolemic individuals, hypertension must be assigned a substantially greater role in the pathology of cerebroatherosclerosis.[23,24]

Diet - Diet continues to be implicated in the etiology of atherosclerosis. In a Seventh-Day Adventist group, among 466 adults, with the exception of those under 25 years of age, the non-vegetarians had higher serum cholesterol values than vegetarians.[25] Several species responded differently to added dietary cholesterol and even individual animals were different within a study.[26,27] It is difficult to predict specific results in one species from data obtained in another species. The rate of cholesterol esterification in rat plasma was increased in pantothenic acid (PA) or choline deficiency, decreased by inositol deficiency and unaffected by pyridoxine deficiency.[28] It has been reported that the M. irus monkey species develops moderate serum cholesterol elevations and coronary atherosclerosis without concomitant elevation of triglycerides on a diet only moderately high (1.4%) in cholesterol. This animal (primate) may serve as a model system to study development of coronary atherosclerosis and its sequelae.[29]

In order to illustrate the different effects of carbohydrates in different species in atherosclerosis studies, one investigation with

rabbits on a cholesterol free diet showed that among glucose (G), sucrose (S), hydrolyzed starch (H), or starch (T), glucose was the least and starch the most atherogenic. Serum cholesterol was elevated in this order of diet, T>H>S>G.[30] Sucrose (S), pregelatinized potato starch (H), glucose (G), and fructose (F) in cholesterol fortified diets in rats produced cholesterol levels in this order, S=F>G>H.[31] In another study with rats, feeding corn starch or other modified starches did not result in cholesterol lowering.[32] In young men, no differences in serum cholesterol or serum phospholipid could be attributed to the carbohydrate - either sucrose or starch - of the diet.[33] Contrary to the opinions of many, the carbohydrate may not be a controlling factor in the metabolism of many animals in this type of study, especially since wide variations of results are obtained under different conditions and in different species.

The role of fatty acids in the etiology of atherosclerosis is far from clear. It has often been suggested that excess saturated fats or carbohydrates in the diet are related to the development of atherosclerosis. Animals that live free in the woodland areas where they eat a great variety of foods including nuts, berries, seeds, etc., get a more oil-rich diet and have considerably more polyunsaturated fatty acid in their tissues than animals in captivity.[34] Fatty acid analysis was made of abdominal sucutaneous adipose tissue of men that died suddenly and the coronary disease evaluated under standardized procedures. The analyzed fatty acids listed in order of their relative contributions to the extent of the disease are stearic, lauric, palmitoleic, myristic, and linoleic.[35] In young men, substitution of safflower oil for butter in the diet reduced serum total cholesterol and increased the fecal excretion of the end products of cholesterol metabolism, which was of sufficient magnitude to account for the lowered cholesterol levels.[36] In another vein of thought, hard insoluble material has been isolated from the intima of atherosclerotic aortas of humans after autopsy. This material was compared (favorably) with oxidized cholesteryl arachidonate,[37] both in fluorescence and general appearance. Lipid peroxidation may be involved in atherogenesis, and thus polyunsaturated fats, plus catylysts such as copper, might enhance the disease.[38]

The effect of stress on the serum cholesterol level and on the atherosclerotic process varies with the kind, intensity, and duration of the stress condition. Exercise stress (physical exertion) has been reported to have prophylactic, as well as therapeutic, implications in atherosclerosis, whereas, emotional stress produces a variety of responses. Physical exercise may retard development of atherosclerosis by increasing the removal of cholesterol by oxidation.[39] In man, immediately after running exercise,[40] serum cholesterol value may be increased but decreases by 12 hours. Cholesterol levels of men who became unemployed rose and then showed a drop later when they found work. Cholesterol in the blood apparently can vary with the duration and severity of the emotional stress.[41]

Among the hormones that have been shown to have some relationship to cholesterol metabolism, atherosclerosis, and related cardiovascular disease, estrogens maintain a position in the focus of research.[42] Oral

contraceptives also have been studied for their effects on cholesterol metabolism.[43] Large doses of Enovid reduced total cholesterol in plasma and cholesterol ester in plasma and adrenals of male and female rats. "The concentration of linoleate and arachidonate was significantly decreased in plasma sterol esters."[44] In contrast, ethynodiol diacetate (1 mg) - mestranol (0.1 mg) combination in human females increased plasma triglycerides and cholesterol;[45] ethinyl estradiol and sequential progesterone in human females caused a slight drop in serum cholesterol while triglycerides increased.[46] Caution has been voiced on the increased use of estrogens as a chronic treatment in men or as a replacement therapy in menopausal women.[47]

The thyroid appears to be involved in cholesterol metabolism--hyperthyroidism relates to lower serum cholesterol, whereas, hypothyroidism relates to elevated serum cholesterol. In myxedematous or hyperlipemic patients, thyroid hormone induced a fall in serum cholesterol level.[48,49] While in vivo studies show that thyroid hormones lower serum cholesterol by increasing catabolism and excretion, in vitro studies, in which cholesterogenesis was measured, clearly showed that T_4 and T_3 inhibited cholesterol biosynthesis.[50] One author believes there is "failure of thyroid function with advancing age and in the presence of severe atherosclerosis."[51] In human postmortem evidence "there appeared to be a correlation between thyroid deficiency and atherosclerosis. Therefore, treatment of atherosclerosis with thyroid seems rational."[52] In spite of these observations, there are many investigators that seem to believe the use of thyroid aggravates rather than ameliorates atherosclerosis. More definitive studies on thyroid hormone effects are essential. Possibly, just normalizing thyroid function from either hypo - or hyper - is the desired endpoint, rather than creating hyper-function in place of hypo-function or vice-versa.

In studies with rats, intravenous injection of insulin and ^{14}C-labeled substrate (glucose or acetate) "led to much greater incorporation of these substances into aortic lipids than when the substrate alone was injected."[53] Insulin may play a major role in the pathogenesis of atherosclerosis through its tissue lipase inhibiting action. Carbutamide, an oral antidiabetic agent, strikingly increased atherosclerosis in cockerels.[54]

Evidence on the relationship of obesity to atherosclerosis has been conflicting and inconclusive. From the International Atherosclerosis Project,[55] results indicate no obvious association between extent of atherosclerotic fatty streaks or raised lesions in either the coronary arteries or the aorta and any measure of body weight, height, or obesity.[56]

Somewhere in the mosaic of atherosclerosis, vascular sensitivity and reaction resembling an allergic response may occur. Hypertension of renal origin may be paralleled by the contribution of some component by the kidney (or by some sources excited by the hypertension or injured kidney) that causes a predisposition of the arterial wall toward atherosclerosis. Vascular injury of the "serum sickness" type from vascular

hypersensitivity was found extremely effective in potentiating atherogenesis.[57] Studies in this area in rabbits have shown that repeated injection of a foreign serum protein caused lesions in the large coronary arteries resembling human coronary lesions.[58] A rather interesting report on a theory of atheroma formation stated that athermatous lesions were found on the luminal surfaces of Dacron and Teflon prostheses placed into the thoracic and abdominal aortas of lipemic rabbits and dogs. It was suggested that these lesions evolve from the deposition of platelets, fibrin and monocytes on the surface of the prostheses.[59] An increased platelet adhesiveness appears present in atherosclerosis and hypertensive cardiovascular disease.[60] The role of platelets in thrombosis and atherosclerosis has been reviewed. The authors concluded that even though there is good evidence that mural thrombi play a significant role in the development of the later stages of atheroslerosis, the function and mechanism of action of the platelets remain undefined. Apparently areas of vascular injury are predilecting sites for the early lesions.[61]

Prophylaxis - Prevention of atherosclerosis and coronary heart disease depends on identification of the disease prone individual and attacking the individual's problem. However, dietary measures, while not hurting in any way those that are not more obviously prone to coronary heart disease, would have a good chance of offering some protection to the large unidentified number almost certain to develop the disease. The Prudent Diet of the Anti-Coronary Club Study Project--nutritionally adequate, palatable, and composed of American foods available in every community-- was acceptable to free-living ambulatory subjects, lowered serum cholesterol, lowered serum triglycerides and appeared to reduce the number of new clinical coronary artery disease events among the test participants compared to expected events.[62] Leren has described his results in bringing about plasma cholesterol reduction by diet.[63] The National Diet-Heart Study Report has been published, showing a fall in serum cholesterol, serum triglycerides, and blood pressure. (Smoking habits also changed, probably due to greater health consciousness from being in the diet study).[64]

Therapy - Since hypercholesterolemia and hypertension are more easily determined in the living than is the diagnosis of the atherosclerotic condition, therapy for the disease depends on diagnosis by inference in most cases. Therefore, treatment of elevated plasma cholesterol levels and elevated blood pressure by drugs and other measures is interpreted as therapeutic measures for atherosclerosis. Indeed, many studies have been reported indicating relief of a variety of symptoms when the above two parameters have been normalized. (However, treatment of hypertension does not fall under the scope of this chapter). In hypercholesterolemia, dietary measures have been applied to both prophylactic and therapeutic purposes and include reduction of intake of cholesterol-rich foods, high-fat foods, and total caloric level.

Even though control of exogenous cholesterol is strict, it may not produce the desired decrease in circulating sterols. Drugs and other measures may be required. Nicotinic acid and its derivativatives continue to be reported to be effective serum cholesterol lowering agents,[65]

by enhancing cholesterol catabolism and possibly reducing cholesterol synthesis.[66] Chondroitin sulfate A was found to have anti-atherosclerotic properties in monkeys, rabbits and rats.[67] Benzyl-N-Benzyl carbethoxyhydroxamate (W-398) is reported to have anti-atherosclerotic properties, as well as hypocholesterolemic effects in rabbits.[68] N-(2-methylbenzyl) linoleamide, depressed plasma cholesterol elevations in cholesterol fed rats and also depressed the deposition of lipids in liver.[69]

Among the many compounds that have been examined for their hypocholesterolemic or cholesterol biosynthesis inhibition properties are diphenylacetylindanedione[70], ethyl 2-methyl-2-phenoxypropionate[71], 1-(2-aminoethyl)-4-(p-chlorodiphenylmethyl)piperazine[72], α,α-diphenyl-β-(4-pyridyl)ethanol[73], 20,25-diazacholesterol[73], 4-amino-1H-pyrazolo[3,4-d] pyrimidine[74], 1-methyl-4-piperidyl bis(4-chlorophenoxy)acetate[75], 4-phenoxyphenol methanesulfonate[76], 2-methyl-2-[p-(1,2,3,4-tetrahydro-1-naphthyl)phenoxy]propionic acid[77], 1-benziloyl-4-benzylpiperazine hydrochloride[72], several substituted 4-biphenylyloxyethylamines[78,79], actinomycin D[80], puromycin[81], candicidin[82], amphotericin B[82], nystatin[82], and neomycin[83,84].

Cholestyramine prevents the entrohepatic circulation of bile acids, and while not affecting serum cholesterol in xanthomatous patients, caused a reduction in size of the modules and a slower rate of appearance of new lesions.[85] Cholestyramine increased cholesterol synthesis and metabolic turnover rate in hyperlipidemic patients.[86] Cholestane-triol lowered serum cholesterol in hypercholesterolemic monkeys[87] and rabbits,[88] but lost its effectiveness in rabbits after 6 months.[89] Sulfonamides have varying effects on liver and plasma cholesterol in rats.[90] Trifluperidol in man caused a significant decrease in serum cholesterol, but also caused accumulation of "quantities of a cholesterol precursor identified as 7-dehydrocholesterol" in the serum.[91]

A factor isolated from liver mitochrondria of 48 hour fasted rabbits or swine produced a marked hypocholesterolemia in the mouse and rat.[92,93]

Ethyl α-(p-chlorophenoxy)isobutyrate (CPIB) or Atromid-S, now being used in the therapy of atherosclerosis and hypercholesterolemia, has a large number of reported effects.[94] Additional effects reported in 1968 have included: Improvement of exudative diabetic retinopathy,[95] but no observable effect on intraocular pressure of Glaucoma,[96] increased oxidation of cholesterol by liver mitochondrial preparations from CPIB-dosed rats,[97] increased rat total liver ubiquinones by blocking its catabolism (synthesis was somewhat inhibited),[98] reduction of plasma FFA in rats,[99] dogs,[100] and humans,[101] reduction in heparin precipitable fraction (HPF) of plasma, and a reduction in erythrocyte sedimentation rate,[102] increase in high density α-lipoprotein,[102] and long term effects - reduces number and severity of attacks of effort angina.[103] It has been reported that the effect of clofibrate on fibrinolysis, platelet stickiness, plasma-fibrinogen and serum cholesterol wears off in 8-9 months in human patients.[103] CPIB also has antifibrinolytic properties.

On the mechanism of action of CPIB as it relates to thyroxine distribution, transport and metabolism in man, "CPIB did not alter the plasma thyroxine iodine, the daily thyroxine degradation rate, or the total thyroxine distribution space." "These findings fail to support the hypothesis that CPIB produces its hypolipidemic effect by displacing thyroxine from its binding proteins and shunting it into the liver."[104]

Several areas in which caution has been sounded on the human use of CPIB involve muscle metabolism,[105] certain enzyme changes,[106] antifibrinolytic properties,[103] effects on cholesterol ester-fatty acid composition changes in plasma,[107,108,109] and the oral contraceptive opposition to the hypocholesterolemic action of CPIB.[110]

Besides the use of drugs and diet to treat hypercholesterolemia, surgical techniques have been applied in the form of bile diversion and ileal bypass or resection. "In dogs subjected to conditions for inducing hypercholesterolemia, reversal has been obtained by bile diversion. The more distal the bile was diverted into the small bowel, the more rapid and significant was the depression of the serum cholesterol levels."[111] Another surgical procedure, ileal bypass, has been found useful in lowering serum cholesterol of animals[112,113] and humans.[114,115] Another allied technique, that of ileal, or distal, resection of intestine has also accomplished lowering of serum cholesterol.[113] Cholesterol absorption is either absent or is greatly diminished when the pancreas is removed or pancreatic secretions are diverted from the intestine in rats and dogs. In man, pancreatic secretions are necessary for proper absorption of cholesterol and the maintenance of the blood cholesterol level.[116]

Comment - Several interesting developments have occurred in 1968, but the atherosclerosis problems are far from solved. A large scale drug test has been started by the National Heart Institute, which should indicate the usefulness of several drugs. In his Duff Memorial Lecture, Page called for "better research"; "reduce the amount of almost trivial, repetitive investigation and increase the number of fresh creative starts."[117]

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Chapter 18. Reproduction

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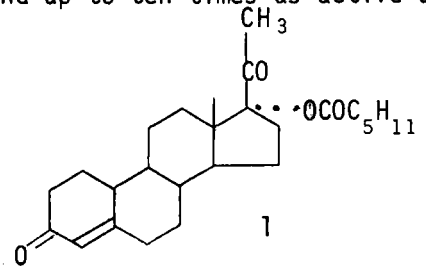
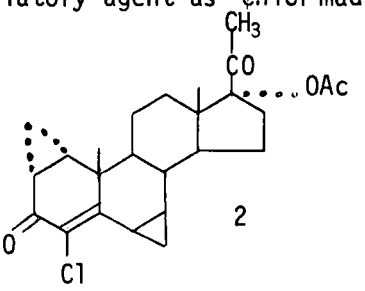
Introduction - In a world seemingly headed for self-destruction through overpopulation the many current advances in medicine may ultimately be of no particular significance. The possibility of overpopulation with its fearsome consequences is no longer being deprecated and is being openly discussed in all the media of publication and on radio and television. Many authorities hope that population control will be voluntarily practiced as the peoples of the world are educated to accept contraceptive techniques. This hope is based on the already observed drop in birth rates in various highly literate modern industrial societies. Many governments are now involved in the once taboo areas of education in contraception, support of reproductive research, the easing of abortion laws, etc. But these efforts seem incapable of averting the inevitable crisis unless the control of reproduction becomes a state function. In the meantime, ideal contraceptive techniques do not exist. But research on both male and female fertility control is being conducted on an ever-increasing scale in the search for fuller understanding of the reproductive process, for newer methods of control, for better synthetic and natural contraceptives, and for new and more convenient methods of using the presently-available hormonal agents.

Steroids - The synthesis of new steroids continued unabated, and many of these are being screened for antifertility activity. Compound 1[1] has an endocrine spectrum superior to its 10-methyl analog. The 4-chloro-bis-cyclopropano compound 2[2] is at least three times as active a progestin and up to ten times as active an antiovulatory agent as chlormadinone (4).

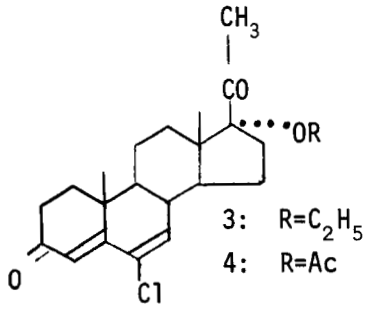


1

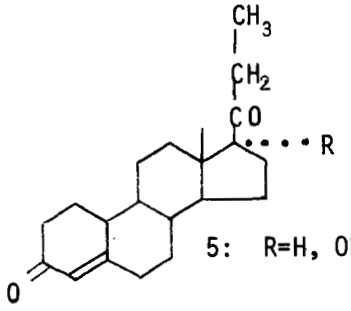


2

The 17-ethoxy analog 3 of chlormadinone (4) has twice the oral progestational potency of the latter, while other 17-alkoxy analogs have much lower activity[3]. Extension of the side-chain by addition of a methyl group, as in 5, to the 17β-acyl portion of potent 19-nor-progestins resulted in a reduction of activity[4]. Progestins without an A-ring oxygen atom have been shown to be active[5]. The compound 6 is one such potent orally active progestin. The trifluoromethyl compound 7[6] is as

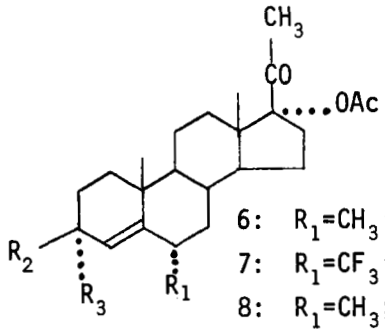


3: R=C_2H_5
4: R=Ac

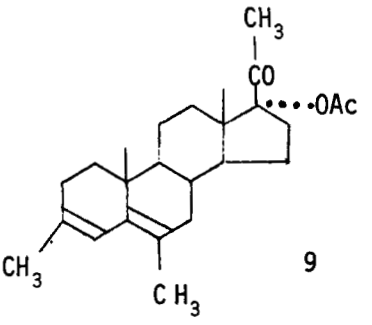


5: R=H, OH, OAc

progestational as 6. The presence of a methyl group at position 3, as in 8 and 9, diminished progestational activity[7].

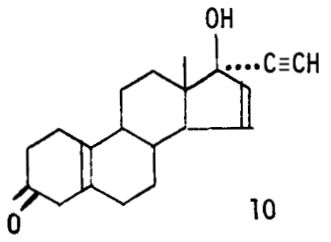


6: R_1=CH_3; R_2,R_3=H
7: R_1=CF_3; R_2=OH; R_3=H
8: R_1=CH_3; R_2 and R_3= CH_3 and OH



9

Introduction of a 15,16-double bond in the 19-nortestosterones 10, 11, and 12 resulted in a four-fold increase of activity in the Clauberg test, as compared with norethindrone[8], but also increased androgenicity in 11 and 12.



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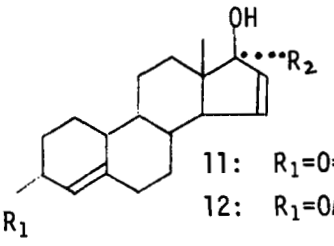
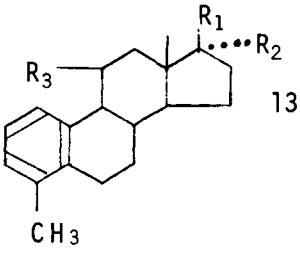
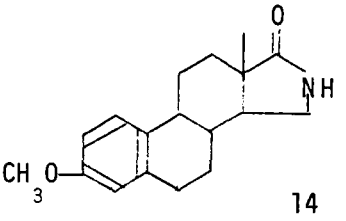


11: R_1=O=; R_2=CH_3
12: R_1=OAc; R_2=CH_3

A series of 4-methylestratrienes 13 showed significant estrogenic activity, precluding their use as lipodiatic agents[9]. These compounds prevented implantation of fertilized ova in rats, at dosages between 140 µg. and 3600 µg. The substitution of nitrogen for carbon in the normal steroid skeleton seems not yet to have improved hormonal actions. Compound 14, 16-azaestrone 3-methyl ether, was found to be a much weaker estrogen than estrone methyl ether and was also antiestrogenic[10].

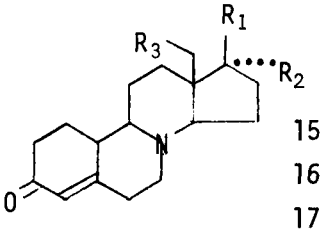


13: R_1=OH, OAc
R_2=CH_3, C≡CH
or R_1+R_2= O=
R_3=OH, OAc

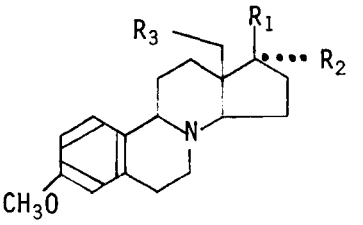


14

8-Aza-19-norprogesterone (15)[11,12] had only 1/40 of the progestational activity of progesterone, and its 18-methyl analog 16[12] had 1/5 the oral activity of 19-norprogesterone. Other nitrogenous analogs 17 and 18 were found to possess much lower activities than their orally-active steroidal models[13].



15: R_1=Ac; R_2,R_3=H
16: R_1=Ac; R_2=H; R_3=CH_3
17: R_1=OH; R_2=H, C_2H_5, C≡CH; R_3=H, CH_3, C_2H_5

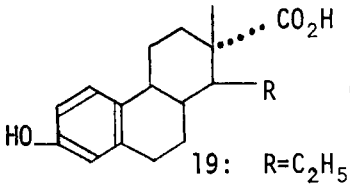


18: R_1, R_2, R_3, as in 17

Studies on female contraception by depot-type injections of steroidal progestins, with and without an estrogen, are still being conducted on a world-wide basis[14,15,16,17]. Many clinical reports on various formulations and dosages, and on efficacy and duration of action have been highly enthusiastic. But "injectables" have not yet been cleared for consumer use. Another approach, still at an early stage, is the subcutaneous implantation of dimethylpolysiloxane capsules containing only neat steroid[18,19]. For several species of laboratory animals it was reported that megestrol acetate in such a capsule inhibited fertility at lower circulating levels than when administered by gavage or injection[19]. A single component "minipill", containing only 0.5 mg of chlormadinone and taken on a daily basis, is being sold in France[14], but little is yet known of its acceptance. In 1966, Ringler[20] had suggested topical application of estrogen plus progestin as a future form of contraception. It has now been reported[21] that steroidal estrogens and two substituted diphenylbenzofurans prevent implantation of ova in rodents when applied topically (post-coital), following Ringler's procedures.

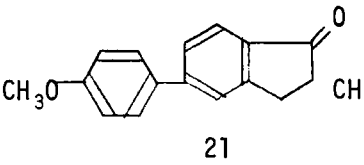
Non-Steroids - Many of the recently reported non-steroidal compounds are variants of compounds already known to affect the reproductive process, but a few new types have been discovered. Substituting a methyl group

for the ethyl group of d-trans-doisynolic acid (19) produced the compound 20, without much change in estrogenicity[22]. An attempt at combining certain features of active compounds led to the indanes 21 and 22, which had only low uterotrophic activity[23].



19: $R=C_2H_5$

20: $R=CH_3$



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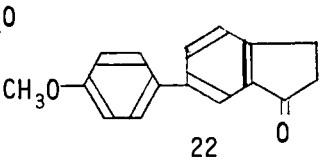
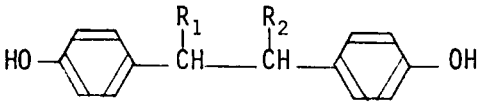


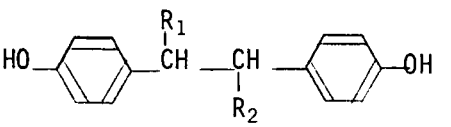
22

A number of isomeric 4,4'-dihydroxybibenzyls 23 and 24 (analogs of hexestrol) have been prepared for the purpose of correlating antifertility activity in the mouse (subcutaneous; days 1-3 or 4-6 post-coital) with other hormonal actions[24]. In general, the antifertility dose closely approximated the estrogenic dose, but 23b was exceptional in showing an antifertility MED of less than 1 µg/day, not explicable as merely an estrogenic or antiestrogenic effect.



23a: R_1, R_2= CH_3 (meso)

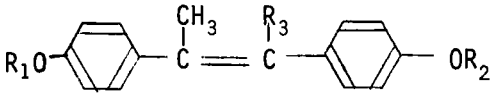
23b: $R_1=CH_3$; $R_2=C_2H_5$(erythro)



24a: R_1, R_2, $=CH_3$ (D,L,DL, threo)

24b: $R_1=CH_3$; $R_2=C_2H_5$(threo)

The synthesis of related compounds 25 and 26 was described late in 1967[25]. It has recently been reported[26] that some of these aminoethers possess antifertility properties roughly paralleling their estrogenicity, and that these activities are lower than for the 23 and 24 types.



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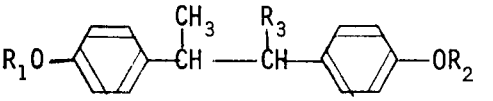
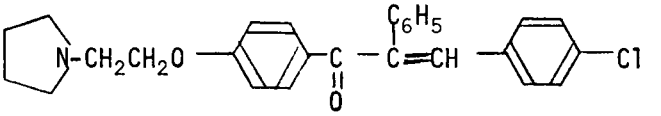


26

For 25 and 26: R_1=H, CH_3, various $R_2NCH_2CH_2$

R_2=various $R_2NCH_2CH_2$

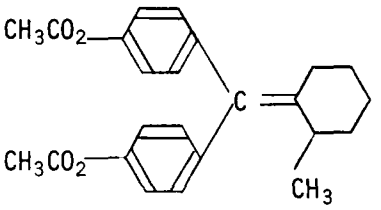
R_3=H, CH_3



27

The 2,3-diphenylacrylophenone 27 showed anti-implantive activity in the rat at a level of 1 mg/kg (intragastric for five days; postcoital)[27]. A large number of analogs with other substituents had much lower activity.

The 2-methyl analog (F 6103) 28 of the previously reported Cyclofenil[28] has been found to be ten times as potent an inducer of abortion in rodents as is Cyclofenil, and is active in many species up to a much later stage of pregnancy. In a very thorough pharmacological study of F 6103[29], it is stated that "A clinical investigation of F 6103's antifertility effects in early pregnancies has been started. Preliminary results suggest that F 6103 can interfere with and eventually interrupt a normally developing pregnancy (Engstrom, pers. comm.)".



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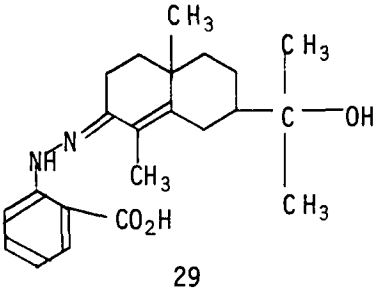
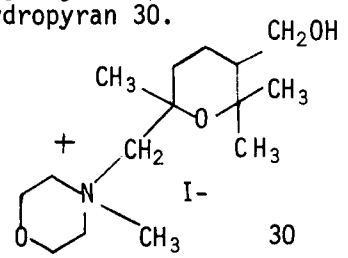
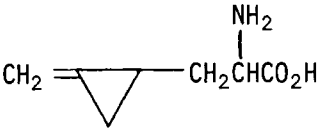


29

The hydrazone 29 was antizygotic in mice at 50 mg/kg, but other derivatives of the parent ketone, carissone (readily available from plant sources in India), were inactive[30]. Another substance said to be active in mice at 50 mg/kg, by unspecified mode of action[31], is the highly-substituted tetrahydropyran 30.



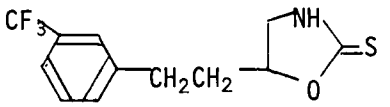
30



31

The rare aminoacid, hypoglycin-A (31) is active in the rat at 15-30 mg/kg (intraperitoneal) up to the sixth day of pregnancy. It is embryotoxic, causing gross retardation of foetal development[32].

The synthesis of many new 4- and 5-ω-arylalkyloxazolidinethiones has produced the compounds 32 and 33, which show antilittering action at 7.5 and 15 mg/kg, respectively[33].



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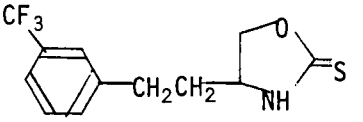
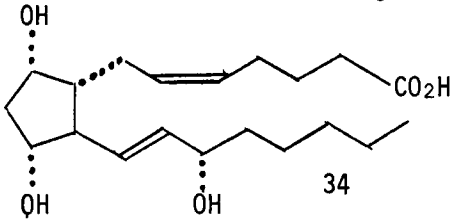


33

Some of the prostaglandins (at least sixteen are now known to exist) play various roles in the complex processes of reproduction. Many have been synthesized and their manifold actions are being studied.

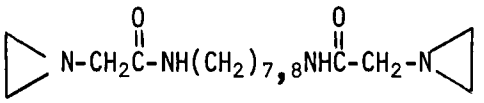


34

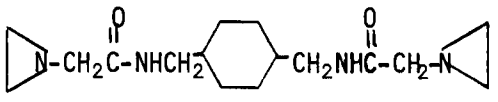
$PGF_{2\alpha}$ (34) is one of the most common of the naturally occurring prostaglandins and has been synthesized[34,35]. A preliminary report[36] on its behavior in the female Rhesus monkey states that when $PGF_{2\alpha}$ is infused intravenously, the corpus luteum disintegrates at an accelerated rate and shedding of uterine endometrium begins. In effect, another menstrual cycle is prematurely started, even after fertilization of an ovum.

Prostaglandins are now also suspected to be involved in male infertility. The number of spermatazoa, their motility, etc., have been correlated with the prostaglandin content of seminal plasma[37]. In this limited study, a low prostaglandin titer accompanied aspermia and oligospermia.

Aziridinyl amides 35 and 36 have been shown to impair the fertility of male mice when injected daily for five days at a level of 20 mg/kg. Seven days after the initial dosing, pairing with a succession of females led to a very low littering rate (or none) for approximately six weeks[38].



35



36

Trimethylphosphate (37) in five consecutive daily doses of 100 mg/kg (male rat; injection or gavage) produced sterility for three to four weeks, by action against post-meiotic germ cells, resulting in intact, motile, but incompetent spermatazoa[39].

$(CH_3O)_3PO$

37

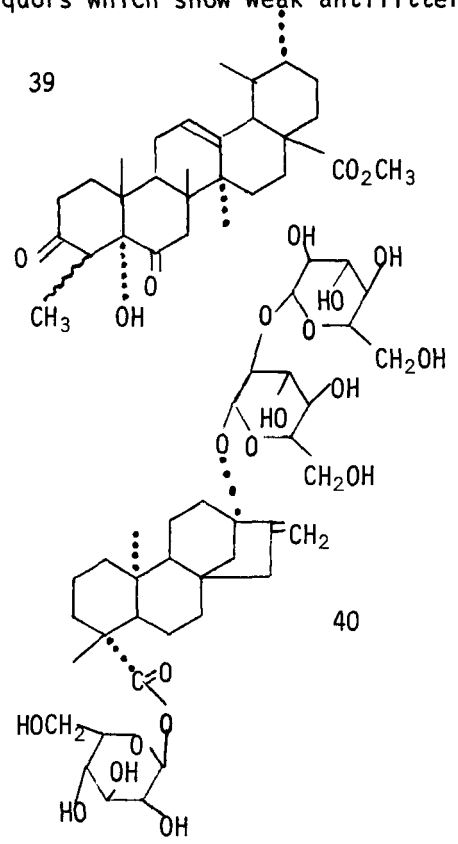
$ClCH_2CHOHCH_2OH$

38

α-Glycerol monochlorohydrin (38) has been reported by Ericsson[40] to produce a very rapid (4-5 days) and yet reversible (7 days) lowering of fertility in male rodents and monkeys, possibly by action on the epididymal vascular system, resulting in normal-appearing but incompetent sperm. Coppola reports[41] that α-glycerol monochlorohydrin has an oral MED of 5 mg/kg/day (for fourteen days) in the rat and suggests that the antifertility effect is a lowering of the motility of vas deferens sperm stores, with the chlorohydrin possibly acting as a metabolic antagonist

in phospholipid syntheses.

Natural Products - The plant Centella asiatica has yielded squeezed liquors which show weak antilittering action in female mice. The glycoside isothankuniside was isolated from the plant and was active at 40-120 mg/kg (diet). Chemical modifications of isothankuniside led to "BK Compound" 39 which is antilittering at 5 mg/kg[42].



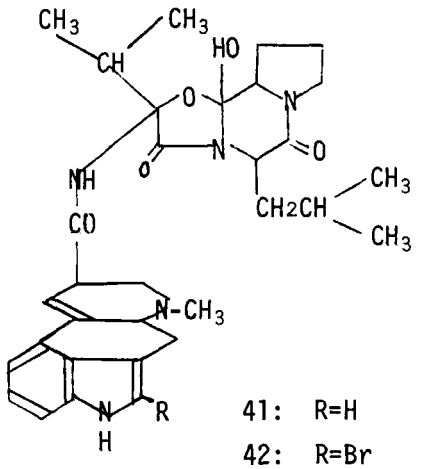
39

It is a practice of Paraguayan Matto Grosso Indian women to drink daily an aqueous decoction from the dry powdered leaves and stems of Stevia rebaudiana Bertoni as an oral contraceptive.

In preliminary experiments, feeding of the crude decoction to adult female rats reduced fertility, and this reduction was still evident two months after dosing had ceased. It was suggested[43] that this contraceptive property is due to Stevioside[44] (40).

40

The alkaloid α-ergokryptin (41) and its 2-bromo derivative 42 interrupt pregnancy in the rat at dosages of 1.15 mg/kg and 0.75 mg/kg, respectively, (subcutaneous on days 5, 10, 15 of gestation)[45]. It was noted that 42 was far less potent than 41 in suppressing lactation, and that bromination probably results in some change in the mode of action of these alkaloids.



41: R=H

42: R=Br

The assiduous bibliographer of botanical antifertility agents may choose to note a reference to Codex postalis[46], but some slight acquaintance with Latin would in this case be recommended.

Biochemical - In a series of papers[47,48,49] Beier has demonstrated the importance of the protein milieu necessary for blastocyst development and implantation and suggested that one of the proteins essential for this process is uteroglobin (M.W. ca. 30,000), which is believed to exist only in endometrial secretions, as demonstrated in the pregnant female rabbit. Alkaline phosphatases are also probably important for the survival and implantation of blastocysts, in that they seem to help maintain the necessary progestational uterine environment[50]. The possibility that male infertility may be due to sperm antibodies is being vigorously investigated in the hope that an immunological form of contraception may evolve[51].

Decapacitation Factor has recently been shown to be present in human seminal plasma, and this suggests that capacitation of sperm may also be required for fertilization in the human[52].

Reviews and Meetings - An excellent review is that of Swyer on "Fertility Control: Achievements and Prospects"[53]. Two highly pertinent reviews by the World Health Organization seem only to have become available recently. These are "Clinical Aspects of Oral Gestogens"[54] and "Mechanism of Action of Sex Hormones and Analogous Substances"[55] . An article entitled "Pharmacologic Alteration of Steroid Hormone Functions"[56] included a section on reproduction and contraception. The two feature articles by Kiefer[57] fully examined the problem of overpopulation in terms of philosophies, prospects, present activities and possible future actions.

The Sixth World Congress of Fertility and Sterility met in Tel Aviv, Israel, in May 1968—there were few reports on basic research but there were many clinical reports on all forms of contraception and on male and female infertility [58,59].

The first annual meeting of the Society for the Study of Reproduction was held at Vanderbilt University, Nashville, Tennessee in August 1968 and was devoted to reports of individual research efforts. A new group called the Population Crisis Foundation of Texas has been formed with the objective of supporting research in all areas of contraception[60] .

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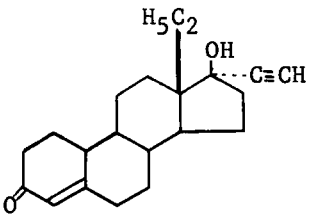
Chapter 18. Steroid Hormones and Their Antagonists

Romano Deghenghi, Ayerst Laboratories, Montreal, Canada

Progestational Agents - The proceedings of a London symposium on norgestrel (1), the latest entry in the antifertility field, has appeared[1] and so have details of its continuous use in microdose form (50-75μg/day)[2] or, in combination with ethynyl estradiol, on alternate days only[3].



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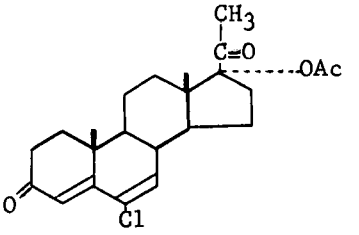
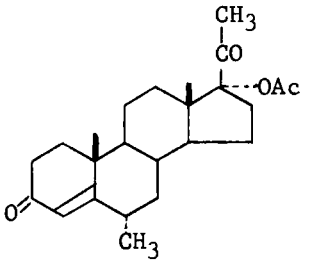


2

An autoradiographic study[4] of in vitro DNA synthesis of the endometrium of women on a continuous low dose (500μg/day) of chlormadinone acetate (2) did not reveal differences from a control group, thus appearing to demonstrate normal hypothalamic-pituitary-ovarian function in the treated group.

Further reports on the antifertility long term effectiveness of injectable medroxyprogesterone acetate (3) (150 mg) in postpartum women[5] and of dihydroxyprogesterone acetophenonide (4) with estradiol enanthate (150 mg + 10 mg)[6], have been published.



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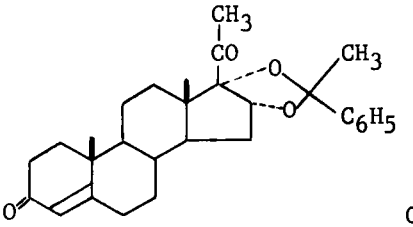

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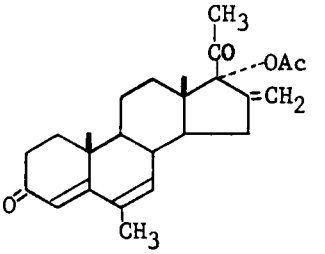
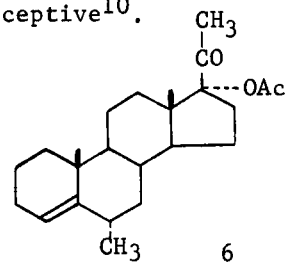


5

A study in monkeys and other animals of hormone-releasing intrauterine devices, consisting of melengestrol acetate (5) incorporated in Silastic material showed that the primate uterus can absorb the progestin from the IUD[7].

An interesting report on the use of a progestin (chlormadinone acetate) (2), in doses of 20 to 30 mg/day for the relief of postmenopausal symptoms has appeared[8]. The rationale was to depress the high level of gonadotropins characteristic of the age and apparently related to the disturbances.

The synthesis of a large number of 3-desoxysteroids was published by a Syntex group[9], followed by an evaluation of a sequential combination of mestranol and 3-desoxy-17-acetoxy-6α-methyl progesterone (6) (ORF-1658) as oral contraceptive[10].



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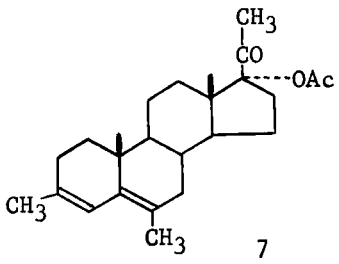
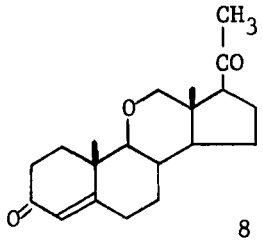


7

The 3,6-dimethylderivative (7) is weakly active as a progestin[11]. The synthesis of the interesting 11-oxaprogesterone (8) was reported[12]. The product has approximately twice the ovulation inhibiting activity of progesterone and 1/6th its potency as measured by the Clauberg test[13].



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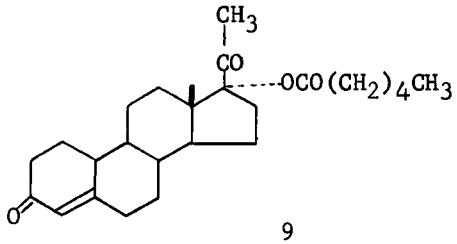


9

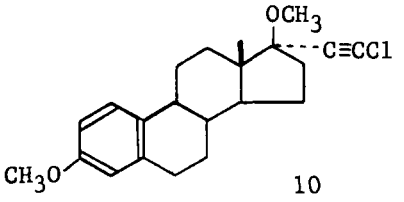
A detailed account of the long acting 19-nor-17-hydroxy progesterone caproate (9) (Gestonorone caproate) appeared in print[14]. Further data on the veterinary use of melengestrol acetate (5) as ovulation control agent in cattle are now available[15]. In animals on melengestrol acetate, estrus and ovulation could be induced with the potent estrogens, estradiol cypionate, estradiol-17β or carbestrol.

The main site of action of several oral contraceptives appears to be the hypothalamus or a higher brain center and not the pituitary, according to a study in rats[16].

A monumental review on progestational agents appeared in press[17].

Estrogens - An extensive review on the chemistry of steroidal estrogens has been published[18]. The relative estrogenicity of a series of oral contraceptives (combination of mestranol and progestational agents) was measured by the immature mouse uterine weight method[19]. Estrogenicity was attributed, apart from the actual mestranol content, to the conversion of some progestational agents into estrogenic compounds.

A long term study on probability of tumor formation in rats given ethynyl estradiol, BDH 2700 (10) and megestrol acetate has been published[20]. The chloroethynyl compound (10) increased the incidence of mammary adenocarcinoma. A reduced occurrence of tumors was observed with other combinations.



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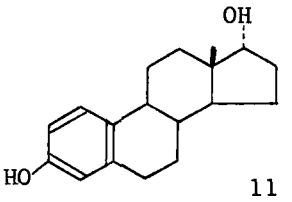


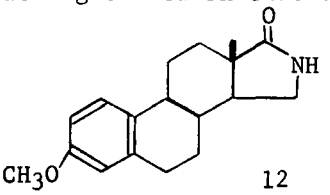
11

Estradiol-17α (11) was shown to displace from the uterus estradiol-17β in ovariectomized rats[21]. A possible inhibition of estrogen-induced uterine responses by analogous 17α epimers is of practical significance because of their presence in conjugated estrogens (equine) of wide therapeutic use.

The dose of ethynyl estradiol that consistently inhibits ovulation in humans is 0.1 mg according to a clinical study based on endometrial biopsy data[22].

A clinical and laboratory re-evaluation of estriol has been published[23].

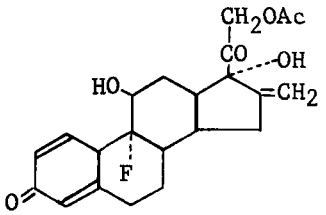
A Japanese study of the metabolic fate of 3-desoxyestrone in man has been preceded by the synthesis of potential metabolites[24]. A statement regarding the usefulness of 3-desoxyestrone as a lipid-shifting drug with no undesirable feminizing effect is apparently at variance with earlier[25] pharmacologic data on this substance.



12

The azaestrone derivative (12) was found to be a weak estrogen capable of antagonizing the properties of its carbocyclic analog estrone methyl ether[26].

Corticoids - Extensive data on the chemistry and pharmacology of 9α-fluoro-16-methylene prednisolone acetate (13), Fluprednyliden-21-acetate, have appeared[27,28,29].



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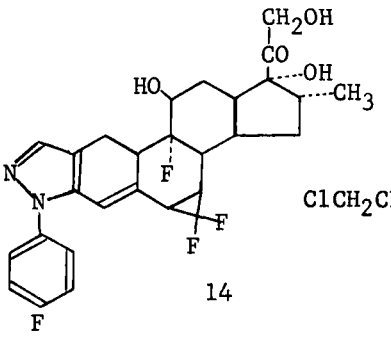

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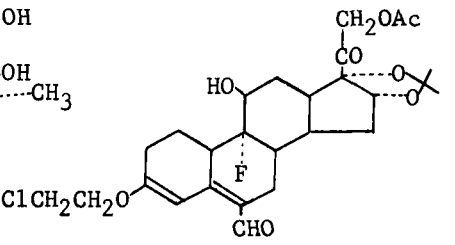


15

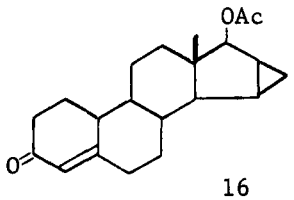
The synthesis of 6,7-difluoromethylene corticoids was published[30]. The most potent of the compounds reported is the 6,7β-adduct (14) (thymolytic assay). Both α and β-face difluoromethylene adducts potentiate corticoid

activity, a fact inconsistent with an anti-inflammatory receptor site which requires binding to rings A and B of the steroid molecule.

Fluorformylon (15) was shown to be active at low doses in protecting mice against fatal anaphylactic shock (experimental allergy)[31].

Molecular orbital calculations on models have provided the intergroup distances which may explain how cortisol functions as an antagonist in the inflammatory response to histamine and serotonin[32]. The distance between the 3-oxo and 11β-hydroxyl group, e.g., approximates the internitrogen distance previously calculated for serotonin.

Androgens - Anabolics - High anabolic/androgenic ratio was reported for the 15β, 16β-methylene adduct (16)[33]. The total synthesis and biological (anabolic/androgenic) activity of compounds related to (17) (d,l racemate) was published[34]. The preparation of 8-azasteroids and their biological activity has been described[35]. These products are generally less active than their carbocyclic analogs. Compound (18) has approximately 1/100th the androgenic potency of testosterone propionate and even less anabolic properties.



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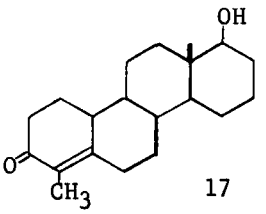

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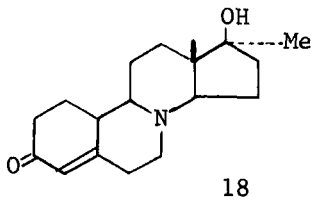
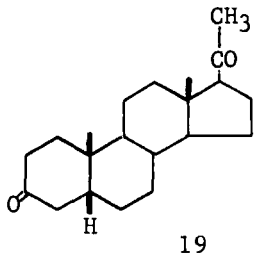


18

A new radiographic technique for the study of anabolic steroids has been described[36]. The dimensions of rabbit muscle are measured photographically after i.m. injection of a radiopaque contrast medium.

Steroids Active on Central Nervous System - The anticonvulsant activity of steroids related to 5β-pregnanedione (19) has been reported[37]. Addition of functional groups tends to decrease the activity. The synthesis of (20), which in humans had sedative properties similar to meprobamate, has been published[38].



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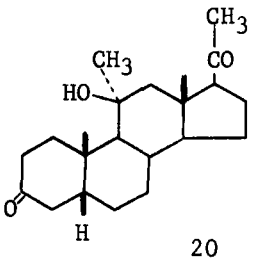

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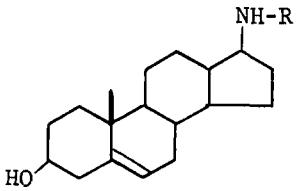
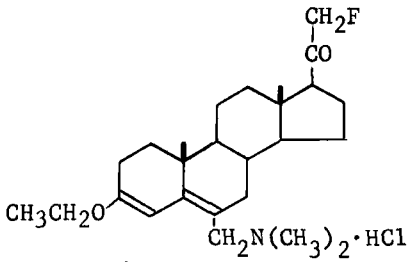


21

Condensation products (21) of 17β-aminoandrost-5-en-3β-ol with several amino acids showed antidepressant activity in mice comparable to the standard amitriptyline[39]. SC-17599 (22) is more potent than codeine or meperidine as an analgetic compound in several assays[40].



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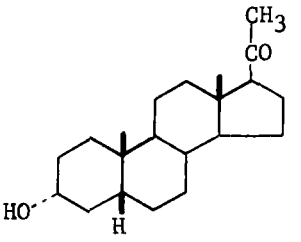
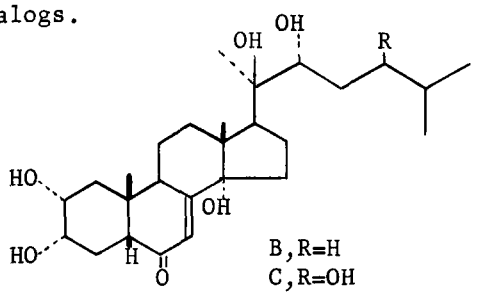


23

A structure-activity relationship of sixty-two steroids as hypnotic agents, and the observation that a few 5α and 5β pregnane derivatives were more potent than barbiturates when given intravenously in nonaqueous solvents, was published[41]. Epipregnanolone (23) had a minimal anesthetic dose of 2.5 mg/kg in the mouse.

Moulting Hormones - The structure of ponasterone B and C (24), the synthesis of A and its identity with crustecdysone (25) have been reported[42,43,44]. Similar hormones are podecdysone A[45], and the amarasterones[46]. The synthesis of active pregnan-6-ones has appeared[47] and some effects of ecdysone and analogs on the development and reproduction of insects were published[48]. Inhibition of larval growth and development was seen with several ecdysone analogs.



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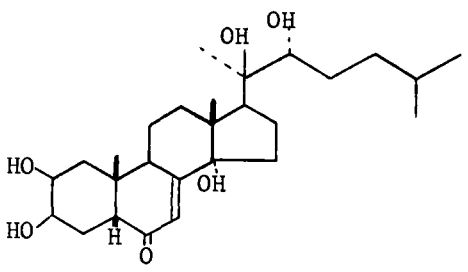
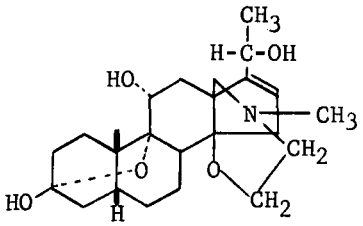


25

Steroidal Cardiotonics - Evidence that a 15β (but not a 15α)-hydroxyl group does not destroy cardiotonic activity and that a 14β-OH function may not be necessary for activity, has been presented[49].

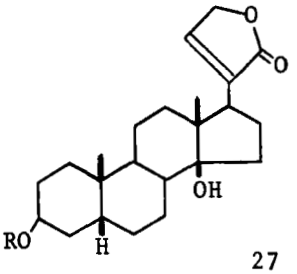
The chemistry and antitumor and cardiotonic activity of strophanthidin derivatives has recently appeared[50]. Lactams were prepared from cardenolides and ammonia or amines, but they were devoid of appreciable cardiotonic activity[51].



26

The structure of batrachotoxinin A (26), a component of an arrow poison from the skin of the frog Phyllobates aurotaenia, has been elucidated by X-ray crystallography[52]. Its activity is 1/500th of the original venom.

Isomeric cardenolides (27), prepared by synthesis, have been described in the patent literature[53]. Two accounts on hellebrigenin (a bufadienolide) with cytotoxic and potent Na-K ATPase inhibition activity have appeared[54,55].



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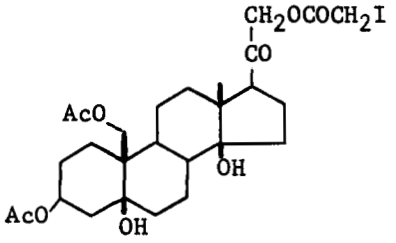
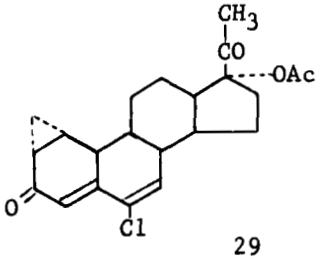


28

The iodoacetate (28) had cardiac activity probably in virtue of its alkylating properties[56].

Structure-activity relationships in a large series of steroidal guanylhydrazones and related compounds were investigated by a Bayer group[57]. The bisguanylhydrazones were found to have inotropic activity.

Antagonists - Objective remissions of carcinoma of the prostate were clinically observed[58] following administration of the antiandrogen cyproterone acetate (29).



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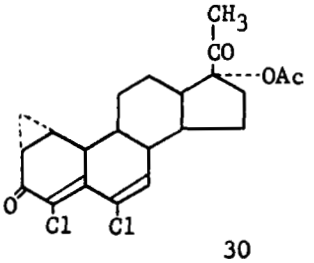

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The 4-chloro derivative (30) was also shown to have pronounced antiandrogen properties[59].

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Chapter 19. Non-steroidal Anti-inflammatory Agents

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Introduction - Useful reviews are available dealing with various aspects of "inflammation" and "anti-inflammatory drugs" in use for treating rheumatoid arthritis, osteoarthritis and gouty arthritis.[1-8] With the exception of gouty arthritis, the etiology and pathogenesis remain obscure. Significant advances towards our understanding of the complex series of events leading to the chronic diseases are expected from studies concerning theories of infection and immune phenomena.[9-12] Rational approaches towards the development of superior drugs (hopefully exerting disease-arresting and curative properties) can only come from such knowledge. Achievement of these goals may lead to the development of prophylactic agents. Advances can also be expected from studies concerning mechanism of action of known anti-rheumatic drugs.

Pharmacological Methodology and Biochemical Considerations - None of the commonly employed acute screens which measure the capacity of drugs to inhibit some form of edema provide a rational approach to the discovery of anti-arthritic drugs. On the other hand, the adjuvant induced arthritis in the rat provides a model of delayed hypersensitivity with an auto-immune component resembling human rheumatoid arthritis.[13] The importance of the circulating lymphocyte in association with the auto-immune component of adjuvant disease has been shown by studies with heterologous anti-rat lymphocyte serum which prevents secondary responses to complete adjuvant.[14] The RNA/DNA ratio of the lymphocyte may be a useful means of measuring immunological activity of an agent.[15] The theory that enzymes released from lysosomes may denature the native constituents of cells of connective tissue and induce the formation of circulating antibodies as part of the normal immune response appears to be a strong possibility in the adjuvant disease of the rat.[13] The increase in serum lysozyme levels observed in the polyarthritic rat may reflect the release of enzymes from leukocytic cells of the inflamed areas into the circulation. Phenylbutazone and indomethacin have been found to be effective in preventing such increases.[16]

Rheumatoid factor may be the immunological link between white blood cells and the body's defense systems.[17,18] Generated 22 S antigen-antibody complex can activate plasma kallikrein and lead to kinin formation and subsequent synovitis.[19]

The 6-sulfanilamido-indazole (6-SI)-induced polyarthritis in old rats is receiving increased attention as a chronic test model.[20] The zinc test in chickens is also being widely investigated.[21] Although the pathogenesis of the syndrome associated with the spontaneous disease in New Zealand

Black Mice (NZB) and hybrid strains of NZB-mice (NZB/NZW) is not established, it is believed to be of an auto-immune nature comparable to systemic lupus erythematosis in man. This disease is a useful experimental model for sophisticated drug testing. The activity of cyclophosphamide has been reported in this model.[22] The induction of acute allergic encephalomyelitis in the Lewis rat provides a system of delayed hypersensitivity especially useful for assessing anti-inflammatory compounds possessing immunosuppressive properties.[23]

The impact of biochemistry on all phases and aspects of the inflammatory process is most impressive. Studies embrace in vitro models, protein synthesis inhibition, protein binding, immunosuppression, metabolism of collagen, specific mechanisms of action and the role of mediators, particularly kinins.

Stabilization of lysosomal membranes has been implicated as an important mechanism of steroidal and non-steroidal anti-inflammatory drugs. Phenylbutazone, flufenamic acid and acetylsalicylic acid inhibit the release of acid phosphatase and β-glucuronidase from isolated rat liver lysosomes incubated in buffered sucrose (pH 7.4).[24] In acidic sucrose (pH 5), acetylsalicylic acid enhances lysosomal enzyme release. Acetylsalicylic acid, indomethacin and phenylbutazone do not stabilize rabbit liver lysosomes.[25] In vitro stabilization of isolated erythrocytes against hypotonic hemolysis[26] and heat-induced hemolysis[27] by non-steroidal anti-inflammatory agents has been employed as a rapid, accurate screening model. The stabilizing potency apparently correlates with the clinical activities of standard anti-rheumatic drugs.

The inflammatory effect of proteases in rats are inhibited by ε-aminocaproic acid, Trasylol® and others.[28] The clinically useful anti-inflammatory drugs inhibit hyaluronidase and β-glucuronidase. Acetylsalicylic acid inhibits acid phosphatase and cathepsins.[29] Gold salts also inhibit synovial lysosomal acid hydrolases,[25,30] presumably by binding with sulfhydryl groups.

The knowledge of lysosomal enzymes has progressed significantly.[31] A new acid pyrophosphatase,[32] two phosphodiesterases[33] and β-aspartylglucosamine amido hydrolase[34] have been identified.

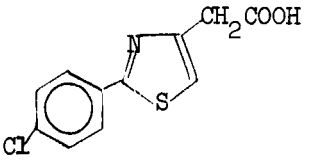
The metabolism of collagen and associated phenomena received continuous attention. Cortisol, oxyphenbutazone and indomethacin but not acetylsalicylic acid, when administered to rats, induce marked and abrupt loss of cutaneous collagen and increased collagenolytic and proteolytic activities in the extra cellular skin compartments. Cycloheximide pretreatment inhibits both effects.[35,36] The problem of lathyrism has been studied.[37-39]

Meclofenamic acid, indomethacin, ibuprofen and acetylsalicylic acid inhibit platelet aggregation. Phenylbutazone is less active in this respect.[40] The anti-rheumatic action of acetylsalicylic acid, phenylbutazone and indomethacin may not be associated with the displacement of

11-hydroxy steroids from plasma proteins in patients with rheumatoid arthritis.[41]

Cellulose sulfate reduces plasma kinin in the rat by depleting plasma kininogen.[42] Polymorphonuclear cells of rabbits produce kinins other than bradykinin.[43] Apparently, corticosteroids do not suppress inflammation by inhibiting kinin formation.[44] Inhibitors of complement, Hageman factor and serum proteases (like Trasylol®) suppress effects induced by the activation of the plasma kinin system via antigen-antibody aggregates.[45,46] The biochemistry of carrageenin-induced edema has been further investigated.[47]

New and Experimental Agents - Arylalkanoic Acids - An impressive number of arylacetic and aryl-alkanoic acids with significant anti-inflammatory properties have been reported during the year. Myalex® (ICI 54450, I) is apparently clinically effective in rheumatoid arthritis.[48] Activity has also been reported for 5-p-chlorophenyl-2-furanacetic acid.[48] Acids II and III have activity comparable to that of phenylbutazone in acute and



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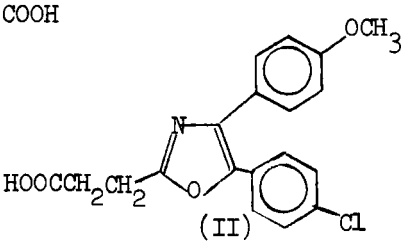

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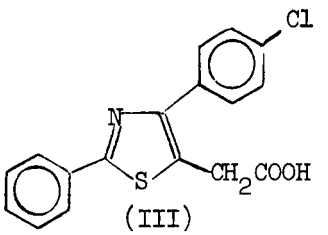


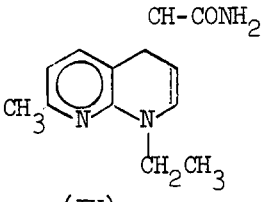
(III)

chronic anti-inflammatory screens and better gastric tolerance is claimed.[49] 4-Isobutylphenylacetic acid (ibufenac, Boots) has shown hepatoxicity in man and has been withdrawn.[50] A clinical study of the corresponding α-methyl acid (ibuprofen, Boots) indicates a potency seven times greater than acetylsalicylic acid.[51]

Anti-inflammatory activity has been claimed for phenylacetic acids substituted in the 4-position by heterocyclic systems;[52] pyrryl;[53] phenyl;[54] and benzoyl.[55] Absorption studies[56] with p-(n-butoxy)-phenylacethydroxamic acid (Droxaryl®) and structure activity relationships in this series have been published.[57] A positive clinical efficacy study on Droxaryl® in chronic rheumatoid arthritis has also appeared.[57]

Additional pharmacological results on 4-(p-biphenylyl)-3-hydroxybutyric acid (BDH 7538) has indicated a general similarity to phenylbutazone.[58] Structure-activity relationships in this series have also been studied.[59]

Anti-inflammatory activity in acute screens has been reported for (IV).[60]



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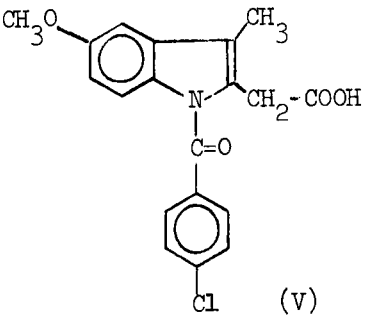

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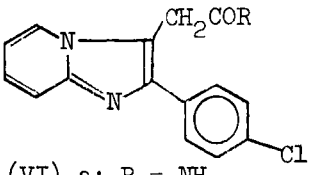


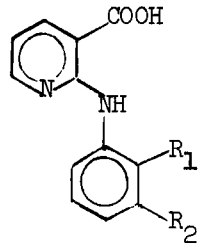
(VI) a: R = NH_2

b: R = OH

Indomethacin Related Agents - A novel synthetic route to indomethacin has been published[61] and applied to variations of the indomethacin structure.[62] Replacements of the acetic acid functionality by longer alkanoic acids,[63] the p-chlorobenzoyl group by a variety of heteroaroyl[64] and cinnamoyl-groups[65] deserve mention. Other variations include esters,[66] heteroaroyl groups such as pyrazinoyl[67] and phenylsulfonyl[68] in place of benzoyl and the replacement of acetic acid by glycolic acid.[69] The indoleacetic acid (V) has also been claimed.[70] No clinical evaluations on these compounds are presently available. The imidazopyridines (VI) are active in the carrageenin edema test in the rat.[71]

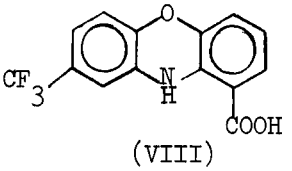
Fenamates and Salicylates - N-(p-n-Butylphenyl)-anthranilic acid has been claimed to have anti-inflammatory activity equal to mefenamic acid.[72] Detailed pharmacology on the tetrazoles related to the fenamic acids has been published.[73] Apparently, these compounds are comparable in activity to the corresponding carboxylic acids.

Much interest has been generated in Nifluril® (VIIa). A clinical study indicates efficacy in cases of ankylosing spondylitis and gout; however, a high incidence (26% of all patients) of gastrointestinal side



(VIIa: R_1=H; R_2=CF_3)

(VIIb: R_1=CH_3; R_2=Cl)



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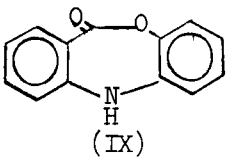


(IX)

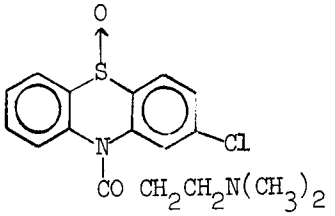
effects is also demonstrated.[74,75] The related analog Sch 10304 (VIIb; CBA 93626) resembles phenylbutazone in carrageenin tests and is claimed to display less ulcerogenicity than fenamic acids and indomethacin.[76] Peak plasma levels are obtained approximately one hour after p.o. administration in man.[77] A clinical study has demonstrated analgesic efficacy.[78] N-Pyrimidinyl anthranilic acids are significantly less active than the

corresponding N-phenyl compounds.[79] The phenoxazine analog (VIII) has anti-inflammatory activity upon s.c. administration but is inactive orally.[80] The cyclic ester analog (IX) has shown local anti-inflammatory properties.[81] A common receptor for fenamates, other anti-inflammatory agents and inflammatory amines has been discussed.[82]

Terpene derivatives of salicylic acid show improved gastrointestinal tolerance but have decreased anti-inflammatory and analgesic activity.[83] Phenothiazines with salicylic acid functionality show only weak anti-inflammatory properties.[84]

4-Acetamidophenyl 2-acetoxybenzoate (WIN 11450) is more effective than acetylsalicylic acid as an antipyretic and in the yeast induced edema screen but is less effective in U.V. erythema.[85] Thymotic acid (2-hydroxy-4-isopropyl-6-methyl-benzoic acid) has been reported active in acute tests but inactive in the chronic adjuvant arthritis model.[86] The hexylcarbonate of salicylic acid is claimed to have less gastrointestinal side effects in experimental animals than acetylsalicylic acid itself.[87] o-Acetoxy-phenylacetic acid has been prepared.[88]

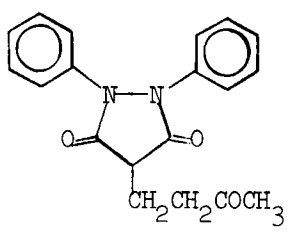
Miscellaneous - Specific amino acids have been studied for anti-inflammatory activity. Thus, the combination of calcium D,L-aspartate/indomethacin has been reported to be effective in primary chronic polyarthritis.[89] Dilauroyl-L-lysine[90] and trans 4-aminomethyl-cyclohexane-1-carboxylic acid (tranexamic acid)[91] have anti-inflammatory properties. The activity of tranexamic acid was not attributed to anti-plasmin action. Detailed pharmacology on SKF 17,910-A (X) has been published. This anti-inflammatory and diuretic agent shares some biological properties with glucocorticoids.[92]



(X)

The anti-inflammatory properties of a series of quinazolinone derivatives have been reported.[93-96]

Ketophenylbutazone (Kebuzone; XI) is reported clinically equivalent to phenylbutazone in rheumatoid arthritis. Reduced fluid retention and possibly less ulcerogenic effects are claimed.[97]



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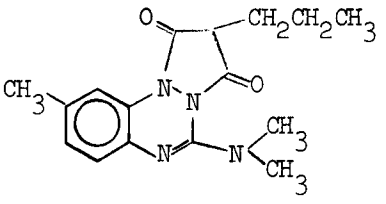

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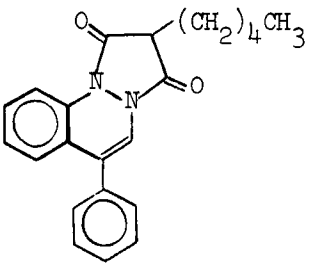
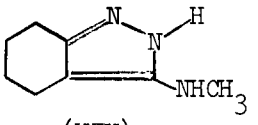


(XIII)

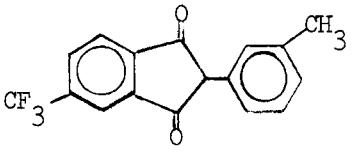
Azapropazone (XII), (Siegfried; Mi 85), has anti-inflammatory efficacy in the clinic.[98,99] Cinnopentazone (XIII), (Siegfried; Scha 306) is ten times more potent than (XII) in the Collier bradykinin test and has bacteriostatic properties.[100] Thienyl substituted phenylbutazone derivatives have been reported as non-toxic anti-inflammatory agents.[101] Clinical studies on 5-butyl-1-cyclohexyl-barbituric acid (BCP) indicate good absorption and prolonged high plasma levels.[102] Pharmacology and structure-activity relationships have appeared.[103] The compound has been introduced on the Japanese market as Paramidin by Takeda.

4-Dimethylamino-1-phenyl-pyrazole has anti-inflammatory, analgesic and antipyretic activity similar to aminopyrine.[104] Tetrydamine (XIV, Poli 67) is in clinical trial as an analgesic/anti-inflammatory agent.[105]



(XIV)

Benzydamine (AF-864) appears to be clinically useful only for the treatment of non-arthritic inflammatory conditions.[106] The acidic indanedione (XV) is active against carrageenin-induced edema and has no effect on prothrombin time.[108] The oxazole (XVI), claimed to be as effective as phenylbutazone in the cotton pellet granuloma test, has been selected for clinical trial.[109] Nimazone (XVII, WIN 25347) is reported to be in clinical trial.[110]



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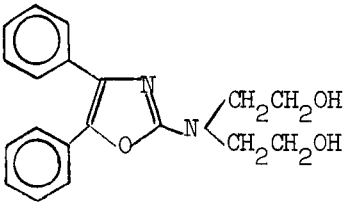

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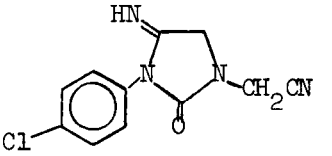
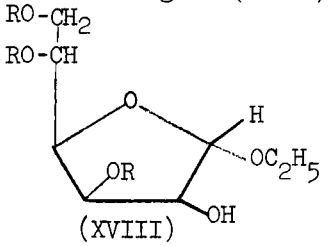


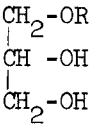
(XVII)

A report on clinical efficacy of Glyvenol® (CIBA 21401-Ba) in rheumatoid diseases is awaited with great interest in view of the unusual structure of this agent (XVIII).[111]



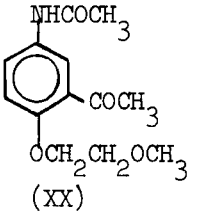
(XVIII)

R= $-CH_2-C_6H_5$



(XIXa): R= $-CH_2(CH_2)_{16}CH_3$

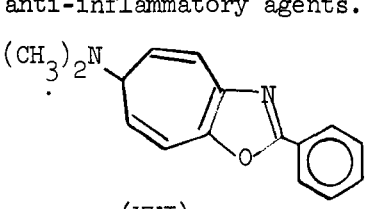
(XIXb): R= $-CH_2-(CH_2)_7-CH=CH-(CH_2)_7-CH_3$



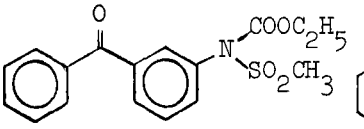
(XX)

The naturally occurring alkoxyglycerols batyl alcohol (XIXa) and selachyl alcohol (XIXb) are orally effective in the granuloma pouch test with a potency equal to hydrocortisone.[112] The human metabolism of the analgesic/anti-inflammatory AP 752 (XX) has been reported.[113]

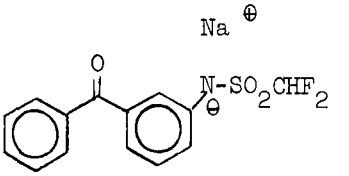
The cycloheptoxazole (XXI) has anti-inflammatory, analgesic and antipyretic properties in animal models.[114] Substituted benzophenones triflumidate (XXII) and diflumidone sodium (XXIII) are in clinical trial as anti-inflammatory agents.[115]



(XXI)



(XXII)



(XXIII)

Vitamin K_1 and Vitamin K_3 have activity in a number of anti-inflammatory models when administered parenterally.[116] α-Tocopherol and linoleic acid inhibit the induction of adjuvant arthritis in the rat.[117] Ormotein, a protein-divalent metal chelate is reported in clinical trial as an anti-inflammatory agent.[105]

Immunosuppressive/Anti-inflammatory Agents - Reports on the apparent clinical effectiveness of immunosuppressive agents in rheumatoid arthritis have greatly stimulated research in this area. ICI 47776 is in toxicity trials with the aim to investigate clinical efficacy in auto-immune diseases.[107] The chemically related tenuazonic acid suppresses histidine-decarboxylase activation and turpentine-induced inflammation in animals.[118] Cyclophosphamide, slow acting but apparently effective in human rheumatoid arthritis,[119] is also active in antipyretic and non-established adjuvant arthritis screens.[120] Podophyllinic acid ethyl hydrazide (Proresid) is reported clinically effective.[121] Cytarabine and other cytotoxic agents inhibit the onset of adjuvant induced polyarthritis in rats.[122] Steroids are even more effective in this respect. The antibiotic rubidomycin is also active.[123] The immunosuppressive and anti-inflammatory properties of 1-amino-cyclopentane-1-carboxylic acid have been recently reported.[124] Azathioprine (Imuran®) reduces corticosteroid requirements of patients with severe rheumatoid arthritis under double blind conditions.[125] A mechanism of action for 6-mercaptopurine has been suggested.[126]

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Section V - Topics in Biology

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Chapter 20. Rational Design of Chemotherapeutic Agents

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Rational approaches to drug design have thus far met with only limited success. The purpose of the present report is to review selected areas of biochemistry and molecular biology applicable to the rational design of chemotherapeutic agents. Emphasis has been placed on novel ideas and procedures which have been developed during the last several years. References were selected to recent publications and review articles that will direct the reader to the original literature.

Structure-Activity Correlations

The complex interrelationships between chemical structure, mode of action and biological activity of chemotherapeutic agents are unlikely to be revealed by a single approach although structure can occasionally be defined at the molecular level by steric and electronic parameters. The problems of distribution, transport and metabolism introduce additional variables when studying the action of drugs in whole organisms. The following approaches have been useful, to a limited extent, in predicting structure-activity relationships.

Regression analysis - Since Hansch's previous review of the subject,[1] he and his colleagues have extended their stochastic approach to structure-activity relationships. This analysis is based, in part, on the hypothesis that sets of congeners acting by the same mechanism on similar receptor sites should have the same log P_o, P being the partition coefficient and P_o the ideal lipophilic character under the test conditions. Constants were reported for barbiturates and for other groups of hypnotics, suggesting guidelines for the design of new CNS depressants.[2] Octanol-water partition coefficients were proposed as a standard reference system for comparative biochemical and pharmacological studies involving hydrophobic bonding.[2]

Similarly, a correlation was reported for the antibacterial activity of 15 groups of drugs acting in such diverse biological systems as intact animals, isolated tissues, bacteria, etc.[3] The authors excluded sulfonamides, which have a different P_o,[4] from this correlation on the basis of their different mode of action.

Molecular orbital calculations - Molecular orbital calculations have been applied to biochemical compounds[5] and to drug design.[6,7] Extended Hückel theory has been used to determine the preferred conformations of such pharmacologically-active molecules as nicotine[8] and pralidoxime.[9] Singer

and Purcell reported molecular orbital calculations on antimalarial drugs and investigated the electronic interactions of quinine, chloroquine and quinacrine with DNA.[10] The experimentally observed binding of chloroquine to the guanine residue in DNA was interpreted in terms of the electron-donating and withdrawing characteristics of these molecules.

Conformational and configurational analogies - Neither regression analysis nor molecular orbital calculations account for differences in the biological activity of stereoisomers but these differences can sometimes be attributed to geometrical factors. The mechanism of action of penicillin[11] and of puromycin[12] has been explained by conformational analogies between these compounds and normal metabolites. Such analogies often suggest similarities between chemically-different classes of compounds more accurately than structure-activity relationships between related congeners. A structural and functional analogy between the ipecac alkaloids and glutarimide antibiotics[13] led to the prediction of the mode of action and amebicidal activity of the indole alkaloid, tubulosine.[14] A conformational analogy between analogs of niridazole allowed certain predictions of schistosomicidal activity among related heterocyclic compounds.[15] Shemyakin et al.[16] observed that the topochemistry of enniatin B, a cyclic peptide antibiotic, was the same as the isomer in which the L- and D-amino acids were replaced by their enantiomeric forms. As predicted, both isomers proved to have identical biological activity.[16] Similar considerations led Strominger to equate D-alanine and D-cycloserine.[17]

Structure-activity relationships often allow the assignment of biological activity to a fragment of the parent molecule. Computer methods of chemical data-storage and retrieval[18] allow such fragments to be identified in other compounds by sub-structure searches.[19] It is also feasible to store three-dimensional data so that molecular models can be visually compared and manipulated on oscilloscope screens.[20]

The chemical definition of drug-receptor complexes would aid in the interpretation of structure-activity relationships. X-ray crystallography has solved selected problems in this area but nuclear magnetic resonance (NMR) techniques offer more general applications. Conformation of small molecules in solution and the nature of functional groups involved in binding to receptors have been determined by NMR.[21,22] High resolution NMR can sometimes distinguish changes in aromatic amino acids found in proteins.[23,24] Proteins that are fully deuterated except for selected amino acids,[25,26] could be used to study interactions of drugs with biologically important macromolecules.

Biochemical Pathways for Selective Inhibition

At present, the cardinal consideration for the rational design of chemotherapeutic agents is the choice of appropriate biochemical pathways for selective inhibition. Recent developments in the chemotherapy of falciparum malaria exemplify the potential advantages of rational design over empirical screening of compounds. Four years of effort and great expense were required to screen 100,000 compounds for antimalarial activity and the results were essentially negative.[27] The presently preferred

antimalarial drugs include a combination of a sulfonamide and a folic acid antagonist. This therapy and its rationale, conceived and experimentally established by Hitchings and his collaborators a decade before,[28,29] has resulted in outstanding success in the treatment of drug-resistant falciparum malaria.[30]

A frequently overlooked benefit of considering biochemical mechanisms is the limitation which can sometimes be placed on the chemotherapeutic potential of a given group of agents. In this regard, studies of the molecular basis of toxicity are equally important to the determination of the mechanism of therapeutic action.

Biosynthesis and function of DNA - Most clinically useful antitumor agents act by inhibiting some aspect of gene replication, transcription or translation.[31] Some inhibitors of DNA synthesis, such as mitomycin[32] or nitrogen mustard,[33] act directly upon the DNA template; others, including methotrexate,[34] 5-fluorouracil,[34,35] 1-β-arabinofuranosyl cytidine (ara-C)[36,37] and hydroxyurea,[38] inhibit DNA synthesis by blocking one or more steps in the synthesis of the deoxyribonucleotide substrates. Still others, such as bromodeoxyuridine (BdUR) or iododeoxyuridine are incorporated into newly synthesized DNA, producing mutations and sensitizing the DNA to radiation damage.[34] The overall mechanisms of nucleic acid synthesis are essentially the same in normal and neoplastic cells. Thus, it seems unlikely that new agents that inhibit the synthesis of DNA will show significantly better therapeutic indices than those currently in use.

Recent attempts to design novel inhibitors of DNA synthesis include the preparation of trifluorothymidine, which has antiviral[39] as well as antitumor activity but, like BdUR, is incorporated into the DNA of animal cells.[40] Ara-C acts as a competitive inhibitor of deoxycytidine and is not incorporated into DNA but is rapidly deaminated to the inactive compound, ara-U.[36] Fox *et al.*[41,42] and Camiener *et al.*[104-106] are attempting to chemically modify ara-C to prevent this deamination and to prepare inhibitors of deoxycytidine deaminase that could be administered simultaneously with ara-C. Ara-A, the adenine analog of ara-C, is active as an antitumor and antiviral agent but is also rapidly deaminated *in vivo*.[36] Schaeffer *et al.* have synthesized a novel class of nucleotides containing a substituted cyclopentyl or cyclohexyl ring at the 9 position which sterically simulates the sugar moiety and inhibits adenosine deaminase.[43,44]

Biosynthesis and function of RNA - Many inhibitors of RNA synthesis, such as actinomycin, act by complexing with DNA and inhibiting its template function in the biosynthesis of RNA.[31] Base analogs, such as 6-mercaptopurine and 8-azaguanine, interfere with interconversions of ribonucleotide subunits and inhibit *de novo* synthesis of RNA.[34] Utilization of these biochemical pathways for the design of chemotherapeutic agents is limited by considerations similar to those discussed for inhibitors of DNA synthesis.

The central role of transfer RNA (tRNA) in the translation of the genetic code suggests that the concentration of specific tRNAs may regulate protein synthesis at the level of translation. Changes in the tRNA population have been identified during hepatic carcinogenesis,[45,46] and

studies from several laboratories indicate that the pattern of tRNA methylation[47] and the abundance of specific tRNAs[48] changes with cellular differentiation and neoplastic transformation. The tRNA species found in tumors may provide a new target for the design of antineoplastic agents if additional studies indicate that these changes are specific to tumor cells.

DNA repair - Efficient mechanisms for excising and repairing damaged regions of DNA exist in normal cells. The biochemistry of these mechanisms is only now emerging from studies in bacteria. An enzyme, "DNA ligase", has been identified which catalyzes the repair of single stranded breaks in DNA.[49] It has been suggested that the cells which are resistant to alkylating agents have acquired more efficient mechanisms for the repair of DNA.[33,50] If this hypothesis is correct, the use of drugs that inhibit DNA repair mechanisms could enhance the effectiveness of alkylating agents and radiotherapy.[51,52]

Protein synthesis and metabolism - Inhibitors of protein synthesis are effective antitumor, antiviral, and antimicrobial agents. Their specific site of action determines whether the desirable chemotherapeutic activities will predominate over the untoward effects of these drugs. The effectiveness of chloramphenicol lies in its inhibitory effect on one of the few enzymatic reactions in protein synthesis that is specific to bacteria and its concomitant failure to affect protein synthesis in animal cells.[53] In contrast, the toxic agents cycloheximide,[54] emetine,[13] and puromycin,[55] inhibit protein synthesis in animal cells as well as in protozoa and yeast. A rational design of antibacterial agents might well concentrate on drugs which act by the same mechanism as chloramphenicol.

A different type of inhibition of protein synthesis in animal cells is manifested in asparagine depletion. The finding that certain leukemias and other tumors have a specific nutritional requirement for L-asparagine[56] constitutes the only established instance of a specific metabolic difference between neoplastic cells and their normal precursors. The suppression of such leukemias by L-asparaginase[57] constitutes the only specific therapy known at present for any type of cancer. Leukemic cells resistant to the effects of asparaginase exhibit substantial asparagine synthetase activities.[58] It thus appears that asparaginase-sensitive tumors have a "metabolic error", i.e. they lack asparagine synthetase.[58] The presence or absence of this metabolic error can be predetermined in vitro by determining the L-asparagine requirement of cell suspensions prepared from lymphomatous nodes, from the bone marrow, or from the buffy coat of the peripheral blood.[57]

Chemotherapy with asparaginase has been limited by the small available supply of this enzyme and by hypersensitivity reactions reported with crude enzyme preparations.[57] The qualitative biochemical difference in the asparagine metabolism of neoplastic and normal tissues suggests other therapeutic approaches. For example, drugs should be sought that selectively inhibit asparagine utilization and biosynthesis in tumors.[58]

Folic acid metabolism - Since the time of Ehrlich, parasitic diseases have

been considered ideal targets for chemotherapy. However, the choice of an appropriate metabolic pathway for a rational approach to drug design is hindered by the paucity of information regarding the biochemistry of these organisms.

The most successful approach to the design of antiparasitic drugs has resulted from the recognition of the sensitivity of protozoa to folic acid deficiency. Sulfonamides prevent the synthesis of folic acid from para-amino benzoic acid in parasites but rarely in man.[28] In addition, a subsequent step in folic acid metabolism, the conversion of folic to folinic acid, can be simultaneously inhibited by depression of dihydrofolate reductase activity with trimethoprim.[28] The dihydrofolate reductase of protozoa is far more sensitive to trimethoprim than that of man.[28,29] This rational design of a combination of drugs is the therapy of choice in drug-resistant falciparum malaria and has been used in the treatment of coccidioisis and toxoplasmosis.[27]

Inhibition of folate reductase[34] accounts for the beneficial effects of methotrexate in certain leukemias and in choriocarcinoma. Unfortunately, the differential effect of methotrexate on tumor cells is not nearly as great as the selective inhibition of folate metabolism in protozoa by trimethoprim. Baker has reported compounds that inhibit dihydrofolic reductase obtained from leukemic cells but do not affect the same enzyme isolated from normal cells.[59]

The "active site" - The design of irreversible enzyme inhibitors directed against the active site of enzymes is a rational approach to drug therapy pioneered by Baker and his co-workers.[60] This approach suffers from the disadvantage of trying to predict a chemical reaction between a potential inhibitor and an enzyme whose structure is defined only in approximate terms. Furthermore, the biochemical rationale on which these studies are based can only be tested after completing the laborious procedure of probing the active site with potential inhibitors. Structural requirements for the ideal inhibitor must eventually be determined by trial and error, although approximations can be made from studies of binding affinities.

Baker's recent studies illustrate some of the problems involved in the design of irreversible inhibitors directed against active sites. A triaminopyrimidine bearing a terminal sulfonyl fluoride group was shown to inactivate the dihydrofolic reductase isolated from L 1210 mouse leukemia but had little effect on the normal enzyme prepared from mouse liver.[59] This important observation was followed by attempts to design other inhibitors operating by covalent bond-formation outside the active site. Although some of these inhibitors met the arbitrary kinetic criteria assigned to theoretically desirable compounds,[61,62] they were not transported through cell membranes and therefore could not be adequately tested for their biological activity *in vivo*.[62] Schaeffer has also tried unsuccessfully to obtain active chemotherapeutic agents by designing irreversible inhibitors directed against the active site of adenosine deaminase.[43,44] The failure of such investigations to produce new compounds of therapeutic value should not obscure the potential of this approach in the rational design of drugs.

Miscellaneous - The enzymes involved in microbial cell wall synthesis[17,63] are obvious targets for the design of antimicrobial agents as a number of useful antibiotics act by inhibiting various steps in this process.[64] The modification of penicillinase-and cephalosporinase-resistant semi-synthetic antibiotics could be aided by knowledge of the enzymology of the β-lactamases.[65]

Glycogen phosphorylase phosphatase is involved in carbohydrate metabolism of S. mansoni and inhibition of this enzyme by niridazole ultimately affects the reproductive system of the female schistosome.[15,66] Phosphofructokinase, which is inhibited by trivalent arsenicals,[67,68] controls the rate of carbohydrate metabolism in schistosomes.[68] The concentration of antimonial must be increased 80 fold to inhibit mammalian phosphofructokinases to the same extent.[69] Kinetic and immunological differences have also been demonstrated between other glycolytic enzymes found in S. mansoni and mammalian tissues.[69]

Piperazine paralyzes Ascaris muscle, acting on this parasite as a myoneural blocking agent, but exerting virtually no myoneural blocking action on mammalian skeletal muscle.[70] Methyridine and bephenium salts produce effects on nematode muscle closely resembling that of acetylcholine.[71] Both compounds possess structural similarities to acetylcholine. It is evident that the neuromuscular system of nematodes, as well as various enzymic pathways in carbohydrate metabolism, offers opportunities for the design of selective antihelminthic agents.

Special Aspects of Neoplasia

Regulatory mechanisms and differentiation - It is apparent that an effective rationale for cancer chemotherapy awaits the elucidation of the molecular basis of neoplasia. It has been suggested that neoplasia is a disease of cell differentiation expressed as an alteration in the control of cellular metabolism and function.[72,73] If certain tumors do arise as potentially reversible aberrations in differentiation, this fact would have important implications for the design of antineoplastic agents. Rather than the conventional emphasis on cytotoxic drugs, regulatory agents should be sought which might permit redifferentiation of the tumor. Instead of designing agents that bind irreversibly to cellular DNA, one should seek drugs that induce specific modifications in gene transcription or translation. This approach is not without precedent if one considers the action of corticosteroids in cancer chemotherapy. There is increasing evidence that these and, perhaps, other hormones exert their primary effects at the level of gene transcription and possibly gene translation.[74]

Cellular kinetics - The rational design of any antitumor agent should consider the cellular kinetics of metabolism and cell proliferation in neoplastic cells. These factors have been briefly reviewed by Skipper[75] and include (a) the generation times of different cell populations, (b) the cell-cycle-phase specificity of certain antitumor agents, (c) the percentage of tumor cell population killed by a given dose, (d) the rate of proliferation of cancer cells, and (e) the rate of host-cell recovery. It has been observed in animals bearing limited numbers of disseminated drug-

sensitive neoplastic cells that approximate matching of a therapeutic regimen to the above requirements may mean the difference between 50-100% "cell cures" and failure even to approach "cell cures" at doses that are equitoxic to the host.

Virus etiology of neoplasia - Extensive studies of tumor cells transformed by DNA or RNA tumor viruses indicate that the viral genome persists in the transformed cell and is at least partially expressed.[76] These important results suggest that, if it were possible to remove the infecting virus, the neoplastic cell might revert to normal. If these interpretations are correct, the rational design of antiviral agents will apply equally to the development of antineoplastic drugs.

Design of Antiviral Agents

The molecular biology of virus replication offers several attractive opportunities for rational drug design. Some recent advances, such as the use of mutant viruses to interfere with viral replication[77] or the administration of interferon, are outside the purview of this report.

Inhibition of viral RNA polymerase - Many compounds have been screened for their inhibitory activity against the RNA polymerase of animal viruses. Several of these possess the desired specificity, inhibiting the viral enzyme but not the DNA-dependent RNA polymerase of the host cell. These agents are selenocystine[78] and a related group of compounds, including gliotoxin, acetylaranotin (LL-S88α), chetomin and the sporidesmins,[79] which possess a common epidithiapiperazinedione moiety.[80] A simple compound containing this structural feature has been synthesized[81] which inhibits the multiplication of RNA viruses[81] and should serve as a starting point for further chemical modification.

Inducers of interferon - The biochemistry of interferon, as discussed in a recent Symposium,[82] suggests several possibilities for the rational design of antiviral agents. In particular, compounds that are not of viral origin are needed as inducers of interferon production by the host cell. Cycloheximide is a chemically-defined small molecule that induces the synthesis of interferon.[83] If its inhibitory effects on protein synthesis, which are probably related to its toxicity, are not responsible for its interferon-producing action, the synthesis of cycloheximide and related glutarimide antibiotics[84] might serve as a starting point for the design of other small molecules that induce the production of interferon by the host cell.

The importance of defining new antiviral agents chemically was demonstrated in recent studies on the antiviral substances isolated from penicillin molds. The antiviral activities of helenine (isolated from Penicillium funiculosum) and statalon[85] (isolated from P. stoloniferum and previously claimed to be an ionic polysaccharide), have been shown to be due to the presence in these molds of double-stranded RNA, a replicative form of RNA found in viral infection.[86] Polyhedral viruses have recently been detected in both strains.[87]

Hilleman and his co-workers have demonstrated interferon production following the administration of reovirus RNA[88] or the replicative form of RNA isolated from E. coli infected with MS2 coliphage.[89] These RNAs are effective in inducing resistance to virus infection in vitro and in vivo. The induction of interferon and the broad-spectrum protection against viral infection conferred by double-stranded RNA suggested the use of synthetic polynucleotides, including polyriboinosinic-polyribocytidilic acid, which possess a double-stranded conformation.[90] Chemically modified RNA may also induce the production of interferon.[91]

Among the chemically-defined macromolecular stimulators of interferon production are the synthetic polymers derived from ethylene maleic anhydride or polyacrylic acid. The structural requirements appear to be a molecular weight of 17,000 or greater and a saturated aliphatic carbon chain with carboxylated groups in alternate or adjacent positions on two out of every four or five carbons.[92] These anionic copolymers are active in vivo in mice against Friend leukemia virus[93] and stimulate the production of interferon in man.[94]

Inhibition of viral mRNA translation - The molecular basis for the action of interferon appears to be an altered ability of the host cell ribosomes to bind viral messenger RNA.[95,96,97] This observation suggested a novel mode for the design of antiviral agents. A model system was devised that would distinguish compounds that inhibit the binding and subsequent function of viral mRNA while only affecting slightly the function of host cell mRNA.[98] Several triphenylmethane dyes, including aurintricarboxylic acid (ATA), showed this selective inhibition.[99] These studies also showed that ATA was unique in inhibiting initiation of viral protein synthesis without affecting the translation of pre-attached mRNA. It remains to be seen whether agents based on this mode of action will be active in vivo.

Viral infection is associated with changes in tRNA.[100] These tRNAs offer potentially exploitable targets for antiviral therapy as they may be essential for the translation of viral mRNA but not of host mRNA.

Nucleic acid analogs - Halogenated nucleotides are effective inhibitors of viral replication by virtue of their incorporation into viral nucleic acid.[101] If virus-specific nucleic acids are identified,[102] an approach to the design of antiviral agents could be suggested since analogs of such bases should not have the same toxic effects as nucleotide analogs that are incorporated into human DNA.

Several unusual "minor" bases occur in tRNA.[103] Most of these are synthesized by secondary modification of bases previously incorporated into a polynucleotide strand. Since the minor bases are critical for the function of certain tRNAs, it is possible that a new type of drug might be developed which interferes selectively with the synthesis of one or more of the minor bases, thus modifying the function of specific tRNAs.

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Chapter 21. Molecular Basis of Drug Action

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Introduction - One of the ultimate goals in medicinal chemistry is to interpret drug-receptor interactions in terms of the attachment of fully defined small molecules inducing fully defined changes in fully defined macromolecules. In some areas of enzyme chemistry this goal is being achieved. The list of proteins the structure of which has been resolved by x-ray crystallography is growing rapidly with the result that the structures of more and more enzymes can be visualized three-dimensionally at resolutions ranging from 1.4 - 5.5 Å[1]. At the same time, Koshland's model[2] of flexible active sites is being strongly supported by the finding of large conformational changes induced by the binding of small inhibitor molecules to the active sites of enzymes[1]. It should be noted that such conformational changes can take place within single macromolecules and do not require the shifting, within a multi-component complex, of subunits with respect to each other, as had been postulated in the model of Monod et al.[3] for allosteric systems.

Where the sequences of biologically important macromolecules are known, one may assume that it will, sooner or later, prove possible to synthesize them. The synthesis of active ribonuclease by classical methods[4] and by the solid-support technique[5] dramatizes what can be done.

However, while extremely complex macromolecules can be assembled, the problem of predicting their conformation from the sequence of their components remains unsolved, nor is it possible to predict what conformational changes attachment of small molecules will induce.

Synthesis of analogs of biopolymers in which single components are replaced by others presents one approach to this problem; numerous such analogs are being synthesized and studied in the field of polypeptide hormones[6,7]. Similarly, the chemical conversion of single amino acids into others within macromolecules is providing insight into the functions of the components of biopolymers. For example, it has proved possible to convert a single serine residue in subtilisin into a cysteine residue[8,9] and to study the modification of the properties of the resulting enzyme.

Information about the stereochemical consequences of replacing single amino acid residues in proteins with others, is also being gathered by studying the molecular pathology of hemoglobin[10]. More than 100 mutant hemoglobins have been found in human blood. It was found that hemoglobin and myoglobin are insensitive to amino acid replacements on the surface but extremely sensitive to even small alterations in the pattern of non-polar contacts, particularly near the heme groups.

While enzyme biochemists and nucleic acid biochemists are working with macromolecules the structures of which and properties of which are increasingly clearly defined, only very few useful drugs act by interacting with biopolymers about which a great deal is known. Medicinal chemists deal primarily with drugs interacting with "receptors" the localization, structure, and topography of which are almost completely obscure.

Even when drugs are known to interact with specific macromolecules, the phenomenon of "allotopy"[11] must be considered. Many vitally important enzymes (and, presumably, other biopolymers) act in vivo while embedded in membranes or attached to subcellular particles[12]. Allotopy refers to the modification of the properties of some enzymes depending on whether they are in solution or bound to a membrane[11]. One approach to this problem has been to study the modification of the properties of enzymes induced by attaching them to various synthetic membranes or other carriers[13]. Work is under way to reconstitute the respiratory chain of mitochondria from its individual components[14]; this approach should also provide a great deal of information about the factors involved in allotopy.

Medicinal chemists and pharmacologists being forced to study drug-induced phenomena so poorly defined that they may be described as "happenings in a garbage can" are limited in the questions about drug-receptor interactions to which they can expect detailed answers at this time.

Questions that can be asked include:

1. Does a given drug act by being attached to a specific receptor?
2. What is the structure and conformation of the drug?
3. What is the structure and conformation of the receptor molecule?
4. What kinds of interaction hold the drug to the receptor?
5. What changes are induced in the receptor by the attachment of the drug?
6. When the same drug molecule interacts with different receptor-macromolecules, do these have identical active sites?

Studies of dose-response curves have been used for a long time[15] to obtain information about the interaction of agonists, antagonists, and partial agonists with receptors. In view of the great interest in conformational changes being brought about by the attachment of small molecules to biopolymers[2,3] a great deal of work has been expended on the interpretation of ligand-saturation curves[3,16,17] observed with catalytic proteins. Attempts are now being made to use the sigmoid

shapes of drug dose-response curves to prove cooperativity in drug-receptor interactions[18,19]. An excellent review of some of the pitfalls that may be encountered on applying the data-plotting methods of enzymology to pharmacological test systems has been provided by Waud[20]. In view of the immense complexity of pharmacological assay preparations as compared to enzymes being studied in solution, Waud's warning "that one cannot safely attach any fundamental significance to the shape of dose-response curves", seems both valuable and timely.

Partition coefficients and their relationships to biological potency provide another tool for obtaining information about drug-receptor interactions. Since the classical work of Meyer[21], Overton[22], and Ferguson[23], researchers have attempted to relate the lipophilic character of drug molecules, as defined by their organic phase-water partition coefficients, to their biological actions. Determination of "thermodynamic activity" has proved useful for deciding whether a given series of drugs does or does not act by being attached to specific receptors. In recent years Hansch and coworkers[24,25,26] initiated a lengthy series of publications attempting to interpret the alterations of biological activity brought about by modifying drug molecules, in terms of a modified Hammett equation:

$$\log (1/C) = k\pi - k\pi^2 + k'' + \rho\sigma$$

The π term, which forms the basis for these calculations, is defined as the difference between the partition coefficient of a parent drug molecule and that of the same molecule carrying a given functional group. While this approach is not without shortcomings[27], it has the great advantage of considering not only the problem of a drug interacting with its receptor, but also taking into account the problem of the drug molecules getting to their receptor. The Hansch group has continued this work during 1968[28,29,30] using a variety of biological systems. Of particular interest seems the surprising suggestion, made on the basis of the measurement of partititon coefficients, that the mode of action of thiobarbiturates differs from that of analogous barbiturates[28]. An attempt to interpret electronic factors in drug-receptor interactions in terms of the principle of hard and soft acids and bases has been made by Cammarata[31].

It seems likely that continuation of the study of partition coefficients will provide a great deal of information about some of the factors involved in hydrophobic interactions. It does not seem likely that this approach will provide a great deal of information about the detailed mechanisms of stereospecific drugs interacting with stereospecific receptors.

The conformation of small molecules is being studied extensively in an attempt to obtain information about the active sites to which they are attached. The assumption that stereospecific, optically active drugs may serve as template molecules of rigid stereospecific

receptor sites[32,33] is being shaken by the consideration that receptor molecules are likely to be flexible. It has been proposed that only a small portion of a drug molecule might be attached to an "affinity site" of a receptor, inducing a stereospecific configurational change of the macromolecule, which may be either helped or hindered by other portions of the drug enantiomer[34]. This explanation might account for the observation that in some series of optically active drug molecules the ratio of the relative activities of the enantiomers may be altered or even inverted as the structure of the molecule is modified.

Analgesics - Since the proposal of an "analgesic receptor site" by Beckett and Casy[33], based on the stereochemical features of narcotic analgesics, a great deal of attention has been centered on the question whether all active analgesics were conformationally related. In view of the known conformation of the phenylpiperidine group in morphine[35] and the postulated features of the "analgesic receptor", tacitly assumed to be rigid, it was proposed[36] that the axial-phenyl conformer of meperidine should fit the receptor more effectively than the equatorial conformer. On the other hand, Ziering et al.[37] suggested that the steric relationship of the phenyl ring was unimportant in conformationally mobile compounds of this type. Syntheses by May[38] and by Smissman and Steinman[39] of analgesics with the phenyl rings fixed in equatorial or axial positions yielded compounds the activities of which were independent of the position of the benzene rings.

Recently, Portoghese et al.[40] synthesized and studied conformationally rigid, diastereoisomeric meperidine analogs. The endo-phenyl epimer was found to be more potent than the exo-isomer. However, the ability of the epimers to penetrate brain tissue was different as well and could be related to differences in their partition coefficients. Slight differences in pK_a and R_f values were also noted. This study is of considerable value in emphasizing the importance of considering differences in the physical parameters of diastereoisomers before interpreting differences in the biological activities of such compounds purely in terms of their abilities to be attached to receptors.

The observation that the more potent enantiomer of α-methadol is derived from the weak analgesic dextro methadone, rather than from its potent levo enantiomer[41] has been confirmed[42]. It seems obvious that the concept of an analgesic receptor site will have to be reevaluated completely and that more work will have to be done to firmly establish the absolute configurations of rigid analgesics using either x-ray diffraction or optical rotatory dispersion spectra[43], while a great deal of information will have to be gathered about the rotational barriers in flexible analgesic molecules.

Nuclear magnetic resonance should be very useful for solving problems in this area. Since the origination of the Karplus equations[44], it has proved possible to solve many conformational and configurational problems[45] by correlating coupling constants between vicinal protons

with the torsional angles between carbon hydrogen directions. The usefulness of this method was exemplified by the recent assignment of the configuration and conformation of all four cocaines by the use of NMR spectra.[46]

COMPOUNDS RELATED TO ACETYLCHOLINE

The remainder of this review will be concerned primarily with the molecular basis of the action of compounds related to acetylcholine. This emphasis is not based on the fact that the mechanisms of action of these compounds are understood on a more fundamental level than those of other drugs. In fact, less is known about their modes of action than about the mechanisms of action of many antibacterial agents. On the other hand, acetylcholine seems unique in the diversity of target-receptors to which it can be attached, while unusually good methods are available for measuring both the "affinities" and "efficacies" of members of this class of compounds.

Conformational Considerations - In view of the great biological importance of acetylcholine and the simplicity of its structure, very numerous analogues of this molecule have been synthesized and studied. It has been known for a long time that cholinergic compounds were highly stereospecific[47]. As in the case of the analgesics, stereospecificity led to the postulate that this class of drugs is attached to rigid, stereospecific receptors[48].

However, while a great deal of attention was centered on optically active analogues related to acetylcholine, until recently, little attention was centered on the conformation of acetylcholine itself.

The proposal was made in 1962[49] and withdrawn in 1963[50] that, in solution acetylcholine assumed a quasi-cyclic conformation involving interaction between the quaternary nitrogen and the polarized carbonyl oxygen of the ester group. Calculations by Gill[51] suggested that, on the basis of steric requirements and interaction energies, acetylcholine should exist primarily in the extended conformation. However, subsequent studies of the structure of acetylcholine in the crystal, using x-ray diffraction[52], and in D_2O solution using NMR[53], indicated that the $-\overset{+}{N}-C-C-O-$ grouping of this molecule is in the gauche conformation in either environment. Thus, acetylcholine forms a quasi-ring structure due to interactions between the quaternary nitrogen and the ether oxygen[52]. This same gauche conformation has now been observed in choline[54], muscarine[55], glycerylphosphorylcholine[56], and lactoylcholine[57]. However, while the torsion angle of the $-\overset{+}{N}-C-C-O-$ grouping shows very little variation in different compounds, the torsion angle of the -C-C-O-C- grouping may vary from 75 to 180° depending on the compound investigated. The suggestion was made recently[58] that "the characteristic gauche conformation is probably associated with the biological activity of nerve amines". The fact that choline (gauche) is devoid of depolarizing activity, while acetylthiolcholine (trans)

is a powerful depolarizing agent is not compatible with this suggestion[59,60].

It was noted that replacement of the ether oxygen of acetylcholine by sulfur or by selenium greatly modified the ability of this compound to induce depolarization[59,60], or to be hydrolyzed by acetylcholinesterase[61]. This, in turn, raised the question whether this alteration of biological activity is due to an alteration in the conformation or due to an alteration in electron distribution.

Studies of the structures of acetylthiolcholine and acetylselenolcholine have now shown that the $-\overset{+}{N}-C-C-B-$ (B = S, Se) groupings of these molecules are in the trans conformation both in the crystal[62,63] and in solution[64]. At the same time, it could be shown that the structures of acetylthiolcholine and acetylselenolcholine were almost identical in either environment. Since the latter compounds were essentially isosteric, it has to be assumed that they must have very similar abilities to fit receptor sites and that the great differences in their depolarizing activities must be due to electronic rather than to conformational factors.

Presumably, the interaction of the quaternary nitrogen and the ether oxygen in acetylcholine which is responsible for the maintenance of the conformation of this compound, may be viewed in terms of a hard acid interacting with a hard base[63] to use Pearson's terminology[65]. Sulfur and selenium being "soft" atoms[65a], such interactions would not be favored in acetylthiolcholine and acetylselenolcholine, which are, therefore, in the extended conformation. The lack of participation of the carbonyl group in such interactions is emphasized by the observation that acetylthionocholine[66], in which the carbonyl but not the ether oxygen was replaced by sulfur, has the same gauche conformation as acetylcholine itself[67] as well as very similar depolarizing activity in the electroplax preparation[68].

While the theory of "induced fit"[2] has been applied widely to possible conformational alterations induced by the attachment of small molecules to receptor macromolecules, in the case of flexible effector molecules, the possibility exists that the conformation of the small molecule might be altered by attachment to the receptor. Recent calculations[69] of the total van der Waals energy of acetylcholine as a function of the various single bond torsion angles suggested that the various conformations with the ether oxygen gauche or trans with respect to the quaternary nitrogen are approximately equally stable, with energy differences of less than 1 Kcal./mole. In view of the observation that acetylcholine and its thiolester and selenolester analogs retain their conformation in the crystal and in solution, the problem of determining the rotational barriers in these molecules is an acute one.

Attempts to obtain information about acetylcholine receptors by the

preparation and study of rigid cholinergic compounds are being continued. For instance, the synthesis of cis and trans 1-acetoxy-2-trimethylammonium cyclopropane has been reported[70]. Muscarinic activity was reported only for the trans compound, which was found to be stereospecific in its action.

Active-Site Directed Molecules Related to Acetylcholine - In view of the widespread interest in compounds designed to form covalent bonds at or near the active sites to which they are attached[71, 72], it is not surprising that there is considerable interest in the preparation of active-site directed acetylcholine analogs. Chiou and Sastry[73] have synthesized fluoro-, chloro-, bromo-, and iodoacetylcholine, as well as the dimethylamino analogs of these compounds. These compounds were found to be substrates of acetylcholinesterase[74] with higher K_m's and lower affinities than those of acetylcholine.

It has been claimed that affinity-labelling of acetylcholine receptors can be accomplished with p-(trimethylammonium)benzenediazonium fluoborate (Tdf)[75, 76]. Phosphorylation of the "esteratic site" of acetylcholinesterase did not interfere with the binding of Tdf to the enzyme. On the other hand, phenyltrimethylammonium, a reversible inhibitor of acetylcholinesterase, protects the enzyme against the action of Tdf, leading to the conclusion that Tdf is a specific labelling agent for the anionic site of acetylcholinesterase. Similarly, it was claimed that Tdf is an affinity-labelling reagent of the acetylcholine receptor site in the synaptic membrane of the electroplax, where it is believed to form covalent bonds with tyrosyl, histidyl, or lysyl residues[75].

An interesting reagent has been developed on the basis of the observation that electrical activity in axonal[77,77a] or synaptic[78] preparations can be blocked reversibly by disulfide reducing agents. If reduction is followed by a reagent capable of reacting with thiol groups, irreversible blockage of electrical activity is induced. Thus, while application of dithiothreitol induces a block of electrical activity which may be reversed by application of an oxidizing agent, dithiothreitol followed by N-ethylmaleimide will block irreversibly. This led to the conclusion that the acetylcholine receptor of the excitable membrane contains a readily reducible disulfide bridge. Reformation of the disulfide bonds can be prevented by alkylation of the thiols formed by reduction. Karlin and Winnik[79], assuming that the disulfide link might be near to the anionic sites of the membrane receptors, prepared p-(N-maleimido)-phenyltrimethylammonium iodide. As expected, the ability of this compound to alkylate thiol groups of the receptor, following treatment with dithiothreitol, was several orders of magnitude greater than that of its dimethylamino analog.

BIOPOLYMERS RELATED TO THE ACTIONS OF ACETYLCHOLINE

Acetylcholinesterase - In view of the crucial roles[80,81] of acetylcholinesterase in the control of electrical activity in the

excitable membranes of nerve and muscle cells and the toxicological importance of acetylcholinesterase inhibitors, the purification of this enzyme has been pursued for a long time[82,83,84]. The crystallization of acetylcholinesterase has now, finally, been accomplished[85]. Unfortunately, the high molecular weight of the protein (260,000) makes it unlikely that the solution of the detailed structure of this enzyme by x-ray diffraction will be accomplished soon. The protein was found to have four subunits of similar molecular weight. The presence of two different kinds of chains could be demonstrated by means of the determination of C-terminal groups[86].

The absolute configuration of Sarin and of related acetylcholinesterase inhibitors has been elucidated[87], an approach that should be potentially useful in obtaining information about the topography of the esteratic site. The claim has been made[88] that optical rotatory dispersion measurements detected conformational changes in acetylcholinesterase in the presence of heat, alkali, acetylhomocholine, and a reversible inhibitor.

In view of the suggestion that acetylcholinesterase and the acetylcholine receptor of excitable membranes were identical[89,90] a great deal of work has been expended to prove the identity or nonidentity of these receptors. Comparative studies measuring the binding of various quinolinium derivatives[91], choline analogs[61], and choline esters[92] have provided further evidence for the nonidentity of the active sites under consideration. The problem of possible cooperative interactions between acetylcholinesterase and the acetylcholine-membrane receptor remains under active investigation[93].

The postulated importance of hydrogen bond formation between the ether oxygen of acetylcholine and the active site of acetylcholinesterase[93a] is made unlikely by the observation that acetylthiolcholine and acetylselenolcholine are bound more tightly to the enzyme than the oxygen analog[61]. Similarly, the observation that cholinethiol, while much less likely to form hydrogen bonds than choline, is a powerful depolarizing agent[60], while choline is devoid of depolarizing activity, makes it very unlikely that hydrogen bonding plays an important role in attachment to the receptor of the excitable membrane. In fact, in the series methoxycholine, methylthiocholine, and methylselenocholine depolarizing action seems inversely proportional to hydrogen-bonding ability[93b].

Because of the difficulties encountered, until recently, in obtaining pure acetylcholinesterase, studies of the kinetics of the interaction of this enzyme with substrates and inhibitors have been one of the primary tools for obtaining information about its active sites. Since the classical studies of Wilson and Nachmansohn[94], the concept of "anionic" and "esteratic" sites has become generally accepted. Evidence has now been presented by O'Brien et al.[95], on the basis of inhibition studies with phosphate and phosphorothiolate analogs, that acetylcholinesterase contains a "hydrophobic patch" near the esteratic site.

This proposal agrees with the observation that chalcogenic replacement of either the side chain oxygen or the carbonyl oxygen greatly modifies the ability of 2-dialkylaminoethyl benzoates or of their trialkylammonium analogs to be bound to the acyl-enzyme[96]. The question, why for some substrates acylation and for others deacylation is the rate-limiting factor during hydrolysis, remains under active investigation[97].

The postulate by Nachmansohn[80] and by Belleau[90] that substrate-attachment is likely to induce conformational changes in acetyl-cholinesterase has not, as yet, been proved conclusively. Since acetylcholinesterase contains subunits and probably forms a structural and functional unit with the acetylcholine-receptor biopolymers of excitable membranes, it seems very likely that even when the existence of conformational changes will have been demonstrated conclusively, it will be difficult to interpret their significance.

Jardetzky and his colleagues[98,99] have shown, by the use of NMR measurements, that in some small molecule-polymer complexes it is possible, through differential rotational stabilization of distinguish-able protons, to obtain information about the factors involved in attaching the small molecule to its "receptor". This method was used to study the interaction of tetramethylammonium ions with the globulin antibody to the hapten determinant phenoxycholine[100]. An increased barrier to the rotation of methyl groups in the bound state could be observed. It has now been claimed that the antibody prepared against the determinant phenoxycholine provides an immunochemical model for cholinergic receptors[101]. Unhappily the specificity of the antibody turned out to be rather low and did not permit differentiation between agonists and antagonists or between muscarinic and nicotinic agents.

A study of the interaction of acetylcholine with serum cholinester-ase, using NMR claimed that the rate of initial line broadening was greater for the quaternary ammonium group than for the acetyl group. This finding was interpreted as showing initial attachment of the substrate to the anionic site of the enzyme, followed by interaction between the acyl group and the esteratic site[102].

The reaction kinetics of the enzymic hydrolysis of the enantiomers of lactoylcholine have been studied. In true and in pseudo-acetylcholinesterase only moderate stereospecificity could be detected[103].

Choline Acetylase - Choline acetyltransferase, the enzyme responsible for the synthesis of acetylcholine from choline and acetylcoenzyme A, was first studied in cell-free preparations by Nachmansohn and Machado[104]. Even though the importance of choline acetylase is as great as that of acetylcholinesterase, much less interest has been centered on the enzyme responsible for the synthesis of acetylcholine than on the enzyme responsible for its hydrolysis, until fairly recently. The properties of choline acetyltransferase isolated from squid ganglia

have been described by Prince[105], while the purification of the rat brain enzyme has been reported by Potter et al.[106] The molecular weight of the rat brain enzyme was found to be about 50,000. Like the squid enzyme it possesses essential thiol groups, unlike the squid enzyme it was reported to be activated by salts.

The report that S-bromoacetyl coenzyme A is a potent inhibitor of choline acetylase[107], was followed by the recent report of a group of styrylpyridine analogs also capable of inhibiting this enzyme[108]. One of these, 4-(1-naphthylvinyl)pyridine is claimed to inhibit choline acetylase without affecting acetylcholinesterase. Quaternization of this compound permits it to inhibit both enzymes.

It seems obvious that during the coming years a great deal of research will be centered on elucidating the molecular mechanisms of the action and control of this important enzyme.

The Acetylcholine Receptor of Excitable Membranes - The isolation of this elusive biopolymer, like the sighting of the Loch Ness monster, is more frequently reported than it is confirmed. At the time of this writing, one can only say that there is good circumstantial evidence that acetylcholine and related compounds, through attachment to a receptor can induce a change in the permeability of excitable membranes to the passage of cations[80,81]. The question whether this receptor is or is not a protein is still being debated; a recent review discusses this problem[109]. The experiments of Karlin[78] and others[77,77a] showing the effects on electrical activity of disulfide reducing agents and sufhydryl reagents are in agreement with the postulate[80] of a protein receptor. However, it seems reasonable to assume that in the acetylcholine-receptor, protein, polysaccharide, and phospholipid components form a structurally and functionally related unit while the possibility of cooperative interaction between choline acetylase, acetylcholine receptor, and acetylcholinesterase cannot be excluded.

In spite of the uncertainties concerning membrane-acetylcholine receptors some questions concerning them have been answered in recent years. Although experiments comparing effects obtained in whole cells with effects obtained with purified enzymes can be misleading, the postulate that "acetylcholine receptor" and acetylcholinesterase are identical proteins have been disproved fairly conclusively by experiments showing that the relative binding abilities of related compounds are quite different in the two systems.

On the other hand, a striking parallelism was detected when the ability of a series of analogues of benzoylcholine and 2-dimethylaminoethyl benzoate, to inhibit electrical activity in axonal and synaptic preparations, was examined[61,110,111]. These results provide preliminary support for the postulate that local anesthetics block similar receptors in axonal and synaptic membranes.

The problem of receptor isolation and purification is very much more difficult than the problem of enzyme isolation and purification. Since an enzyme "does something", its activity provides a criterion of purity. On the other hand, receptors, apart from their ability to "receive" small molecules, have no activity which can be measured readily.

In the case of the acetylcholine receptor, the recent availability of some active-site directed labelling compounds, raises some hope that its isolation will finally be accomplished, although it seems likely that more highly specific--and preferably stereospecific--compounds than those used so far will be required for this purpose.

METHODS OF STUDYING THE INTERACTION OF SMALL MOLECULES AND NON-PURIFIED MACROMOLECULES.

As already noted, medicinal chemists, in contrast to enzyme chemists, are frequently concerned with studying the interaction of drug molecules with macromolecules which are very poorly defined, to put it charitably. This has the very unfortunate drawback that some of the most powerful methods available for studying the detailed structure, and the small molecule-induced conformational changes of biopolymers cannot be applied. X-ray diffraction, optical rotatory dispersion, and circular dichroism have been useful to medicinal chemistry only in isolated instances. Fortunately, some methods not requiring highly purified macromolecules are now becoming available.

Nuclear Magnetic Resonance - Application of this tool provides a powerful method for studying the conformation of small molecules in solution[45,46,53,64] as well as for studying their rotational barriers. For example, use of nuclear magnetic resonance could provide a great deal of information about the conformation of diphosphopyridine nucleotide[112]. Use of this technique has proved useful for obtaining information about the active sites of enzymes, such as chymotrypsin[113], lysozyme[114], or ribonuclease.

It has also proved possible to use NMR techniques for studying the attachment of drug molecules to polymers which are not completely defined and to ascertain what part of these molecules is involved in the resulting complex. In addition to the aforementioned investigation of the binding of tetramethylammonium ions to a globulin antibody[98,99], this method could be used to study the attachment of sulfonamides and penicilline derivatives to proteins[115].

Spin Labelling - In recent years there has been a great deal of emphasis on the preparation and study of various kinds of macromolecular probes. Probes may be defined as small molecules, some measurable property of which is altered as the result of non-covalent attachment to a biopolymer. Fluorescence[116], optical absorption, NMR[117], and electron-spin resonance[118] have all formed the basis for the design of probing molecules. While all of these techniques have their

advantages, the use of spin labels permits the investigation of small molecules and macromolecules which are either optically transparent or opaque, furthermore, since few components of biological systems are paramagnetic, the resonance spectra of free radicals introduced into such systems are essentially free from interference.

"Spin labels" have been defined as synthetic free radicals which may be attached to biopolymers. Due to the alteration of the environment of the labelling molecules such attachment should result in a measurable change of their paramagnetic resonance spectrum. Di-t-alkyl nitroxide radicals, being remarkably unreactive, have been particularly convenient for this purpose and have been used to label a variety of proteins. It proved possible[119] to use spin-labelling to show that the oxygenation of hemoglobin followed the scheme for homotropic allosteric effects proposed by Koshland et al.[16] rather than that of Monod et al.[3]

Attempts are now being made to apply spin labels to biological membranes and to models of biological membranes[120]. For instance, it could be shown by Hubbell and McConnell[121] that 2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPO) could be used to label excitable membrane systems such as the vagus of the rabbit, the walking leg nerve of the lobster, and a muscle membrane. This label was not bound to erythrocytes, mitochondrial membranes, or bovine serum albumin.

This is a very new tool; its potential usefulness would seem to be great.

CONCLUSION

It is becoming obvious that because of the previously unsuspected problems of multi-structure complexes, cooperativity, conformational changes, and allotopy, the problem of drug-receptor complexes is very much more involved than the classical concept of a "lock-and-key" interaction.

On the other hand, there is some modest cause for optimism that the availability of tools for studying the problems of drug-receptor interactions will increase at least as rapidly as the complexity of the problems being studied.

Since we now have methods for studying the detailed conformation of small molecules and macromolecules, studies of the topography of the active sites of receptor molecules through the ability to attach rigid, small molecules, will be facilitated. Isosteric, non-isoelectronic analogs of biologically active molecules provide a useful approach for the study of the identity or non-identity of different receptors and for the study of the bonding forces within the drug-receptor complex[34].

Active-site directed acylating and alkylating agents forming covalent bonds at or near active sites should provide a useful method for labelling biopolymers during purification procedures. On the other hand,

non-covalently attached probes will be useful for yielding information about the dielectric constant at or near active sites, as well as the conformational changes induced by small-molecule attachment.

The immense usefulness of x-ray diffraction needs no discussion. Spin labelling and nuclear magnetic resonance have just begun to be used widely while more and more powerful methods for the isolation, purification, and synthesis of biopolymers are becoming available.

Thus, the hope that the action of at least some drugs can be interpreted on a molecular level within the next decade seems a realistic one.

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Chapter 22. Drugs and Deterrence of Alcohol Consumption

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Introduction - In last year's Annual Reports one of us (A.W.) reviewed "Drugs and Memory and Learning". The present subject constitutes another research and treatment area on the periphery of conventional psychopharmacology and medicinal chemistry. As in the case of memory and learning, many biological facets of alcoholism are abundantly reviewed. The documentation section of the Quarterly Journal of Studies on Alcoholism provides a convenient entry to all current literature. Recently, Koppanyi and Maengwyn-Davies[1] have broadly examined "Drugs Used in Alcoholism Research and Treatment"; the detailed review by Zipf and Hamacher[2] embodies much of the same material. This report will focus more sharply on potential, claimed or actual drug therapy for deterring alcohol intake in the chronic alcoholic. Drugs used in delerium tremens[3], alcohol withdrawal[3], alcoholic coma[3], etc. will not be considered. We shall also avoid the controversial issue of whether antidepressants and tranquilizers[4-7] or hallucinogens[8,9] exert indirect beneficial effects on the excessive drinker. Citations noted above may be consulted for current reviews of each subject.

Aldehyde Dehydrogenase Inhibitors

Disulfiram - First advocated as a therapeutic aid in alcoholism by Danish workers in 1948[10,11], disulfiram continues to occupy a role as an adjunct to psychotherapy and behavior therapy. At the very least, "disulfiram...appears to be a good test of the motivation of the patient for sobriety"[6]. That disulfiram acts by blocking aldehyde dehydrogenase, causing a toxic elevation of circulating acetaldehyde when alcohol is consumed, continues to be generally accepted as its primary mechanism of action. Whether such blockade solely accounts for the clinical manifestations of the disulfiram-alcohol interaction has been questioned. Based on studies in rabbits, it has been suggested that an unidentified acetaldehyde metabolite may be responsible for the hypotension[12]. Because they were unable to demonstrate elevated acetaldehyde levels in mice after disulfiram plus alcohol, other authors[13] concluded that a compound resulting from a chemical reaction of disulfiram and alcohol, "probably a quaternary ammonium base", is responsible for the toxic response. These and other aberrant findings have been considered[14], and attributed to relatively insensitive assay techniques and ineffective disulfiram treatment schedules.

Contributing to the difficulty of unequivocally relating clinical effects to biochemical mechanisms is the fact that disulfiram inhibits several types of enzymes. It irreversibly inhibits many enzymes containing active sulfhydryl groups *in vitro* by mixed disulfide formation, accompanied by simultaneous production of one molecule of diethyldithiocarbamate per molecule of disulfiram[15]. In fact, the stoichiometric

nature of this reaction provides the basis for a sensitive radioassay for sulfhydryl groups[16]. Enzymes that are inhibited by disulfiram in this way include aldehyde dehydrogenase[15], D-amino acid oxidase[15], hexokinase[17], β-hydroxybutyrate dehydrogenase[18], and mitochondrial NADH dehydrogenase[18]. The disulfide linkage in the inhibitor is absolutely essential[15], since diethyldithiocarbamate is inactive unless it is oxidized *in situ* (e.g., by oxidized cytochrome c or methemoglobin) to disulfiram[17].

Although, as noted above, early doubts that circulating acetaldehyde is the cause of the disulfiram-alcohol interaction were probably based on premature conclusions from an inability to detect acetaldehyde buildup, a more serious objection arises from the metabolism of disulfiram itself. In rodents, unchanged drug (disulfide) is not detected after disulfiram administration[19,20]. Instead, efficient reductive systems of the body (*via* glutathione, protein SH groups, coenzyme A, etc.) rapidly convert disulfiram to diethyldithiocarbamate, which is then rapidly metabolized by way of four paths; a) conjugation to the glucuronide, b) oxidation to sulfate, c) decomposition to carbon disulfide and diethylamine, and, to a slight extent, d) reoxidation to the disulfide[19]. Furthermore, only a small fraction of the available SH groups in soluble proteins of liver are bound as mixed disulfides after disulfiram administration[19]. Thus, because of the absence of intact disulfiram *in vivo*, the disulfide type of inhibition of aldehyde dehydrogenase should not occur. Strömme has accordingly concluded that the disulfiram-alcohol syndrome may result from "acute insufficiency of this [glucuronide] detoxication system" because of overloading by alcohol, by diethyldithiocarbamate conjugation, and by the reduced $NAD^+/NADH$ ratio[19]. Species differences may nevertheless confuse the picture. Strömme's disulfiram doses appear low for rats[19] (although not for mice[20]), compared to what is required to achieve detectable acetaldehyde levels *in vivo* with alcohol[14] and inhibition of liver aldehyde dehydrogenase[21]. The fact that other structures which share the property of sensitizing man to alcohol also cause accumulation of acetaldehyde in animals after alcohol, and also decrease aldehyde dehydrogenase activity *in vivo*, tends to support the view that the disulfiram-alcohol syndrome derives mainly from accumulation of excess acetaldehyde. Inhibition *in vivo* of liver aldehyde dehydrogenase in animal studies implies a greater degree of intracellular reoxidation of the inactive metabolite, diethyldithiocarbamate, to the parent disulfide inhibitor[22], when larger doses of disulfiram are administered.

Some experienced clinicians belittle the incidence and severity of side effects from disulfiram[23,24]. Perhaps administration of lower doses than those initially recommended accounts for this conviction, since a substantial, well reviewed[25] literature attests to the occurrence of such undesired effects. Disulfiram itself, especially when given chronically, can elicit aberrant behavioral (even psychotic[26]) and cardiovascular reactions, and the acetaldehyde syndrome which follows co-administration of disulfiram and alcohol (pronounced hypotension, headache,

hyperventilation, nausea, and vomiting, etc.) has proven dangerous as well as unpleasant. The cardiovascular manifestations of this reaction continue to produce sudden patient deaths[27,28].

Benefits which might accrue from a long lasting dosage form of disulfiram have led some European investigators to implant disulfiram tablets surgically[29-31]. Success in prolonging the aversive response to alcohol can thereby be achieved, but not without amplified dangers.

In connection with the behavioral disturbances seen after prolonged treatment with disulfiram, it is noteworthy that this drug, by way of its metabolite diethyldithiocarbamate, inhibits dopamine-β-hydroxylase. Consequently, disulfiram treatment decreases norepinephrine and elevates dopamine concentrations in brain stem and hypothalamus[32]. Inhibition is believed to result from chelation of the copper of the enzyme by diethyldithiocarbamate, a strong copper-complexing agent[33]. Inhibition by mixed disulfide formation with disulfiram may also contribute, since sulfhydryl sites have been reported for dopamine-β-hydroxylase[34].

Microsomal drug-metabolizing enzymes in liver also appear to be inhibited by disulfiram or its thiol metabolite. For example, disulfiram elevates serum diphenylhydantoin concentrations, presumably by blocking its para-hydroxylation[35]. Diethyldithiocarbamate prolongs hexobarbital sleeping time in mice[36], another indicator of antagonism of drug-metabolizing enzymes. Alcohol has been reported to be oxidized in part by a liver microsomal enzyme[37]. If this system is physiologically significant[38], then disulfiram might affect yet another aspect of alcohol metabolism.

Calcium Carbimide - The severity of the acetaldehyde syndrome when disulfiram is used to inhibit aldehyde dehydrogenase, the frequency of side effects induced by disulfiram *per se*, and the claimed general unreliability of disulfiram's effect prompted a search for other drugs without these disadvantages. Citrated calcium carbimide (cyanamide; Temposil), first advocated as a therapy in alcoholism in 1956[39], appeared to fill this need; more recent publications[40-43] continue to assert its usefulness and safety advantage vs. disulfiram, especially with respect to cardiovascular effects. Calcium carbimide has been employed in Canada and Europe for several years, but has never been introduced in the United States. A pronounced thyrotoxic effect (>propylthiouracil) after chronic dosage, not observed after disulfiram[44], occurs in rats[44,45]. The use of calcium carbimide in the treatment of primary hyperoxaluria is based on its aldehyde dehydrogenase inhibiting action, by which conversion of glycolaldehyde to glycollic acid is blocked[46].

Although acetaldehyde is detectable in human and rodent blood after treatment with calcium carbimide and alcohol[39], *in vitro* inhibition of rabbit or bovine liver aldehyde dehydrogenase can not be demonstrated[21], despite an earlier claim to the contrary[47]. Livers from rabbits treated with cyanamide do show a notable decrease in ability to oxidize indoleacetaldehyde. A metabolic product of cyanamide may be the

actual inhibitor of aldehyde dehydrogenase *in vivo*[48].

Antidiabetic Sulfonylureas - The antidiabetic sulfonylureas, e.g. chlorpropamide and tolbutamide, comprise another structural class which interacts with alcohol to produce a mild disulfiram-like reaction[49-51]. Accumulation of acetaldehyde has been observed after tolbutamide and alcohol in man[52], and after chlorpropamide and alcohol in rats[53]. Both sulfonylureas enhance the vasodepressor responses of exogenous acetaldehyde in cats, supporting the inference of decreased metabolism of acetaldehyde[53]. These experiments suggest inhibition of hepatic aldehyde dehydrogenase; however, only weak *in vitro* inhibition has been found[54,55]. Nevertheless, sulfonylureas have recently been reported to inhibit purified aldehyde dehydrogenase noncompetitively[56]. Furthermore, the metabolism of serotonin-^{14}C *in vitro* is diverted from 5HIAA to neutral metabolites[56]. Büttner has suggested that the action of the sulfonylureas in antagonizing acetaldehyde oxidation may result from blockade, in the presence of alcohol, of the reoxidation of NADH to NAD^+, rather than from direct action on the enzyme, as in the case of disulfiram[57]. Such a decrease in NAD^+/NADH ratio should also affect alcohol dehydrogenase activity. A 34% decrease in the rate of ethanol disappearance has been observed in cats treated with tolbutamide[58]. Chlorpropamide, tolbutamide, and phenbutamide have been shown to reduce the preference of selected Wistar rats for 5% alcohol (vs. water); carbutamide and metahexamide (among other sulfonylureas) are ineffective[59].

n-Butyraldoxime - Butyraldoxime was first reported to cause intolerance to alcohol after an on-the-site toxicological study of factory workers exposed to it[60]. The reaction was typical of disulfiram, and a small increase in acetaldehyde blood levels could be demonstrated. Although butyraldoxime was considered "a new cure for alcoholism", subsequent reports of any clinical use are lacking. Recently, butyraldoxime has been found to cause a marked, prolonged reduction in alcohol selection by C57BL mice[61], a strain known to prefer alcohol to water[62]. Interestingly, especially in view of the inhibition by butyraldoxime of alcohol dehydrogenase (see below), attempts have been made to relate the strong alcohol preference of the C57BL strain to its greater hepatic activity of alcohol dehydrogenase[63] and aldehyde dehydrogenase[64]. In DBA mice, which avoid alcohol concentrations preferred by C57BL mice, both enzyme systems exhibit less activity.

Butyraldoxime, unlike disulfiram, exerts virtually no inhibition on hepatic aldehyde dehydrogenase *in vitro*[54,61], a finding seemingly inconsistent with the initial observation in man. On the other hand, a marked decrease in hepatic aldehyde dehydrogenase activity is seen in mice treated with butyraldoxime[61]. Thus, butyraldoxime resembles cyanamide in inhibiting aldehyde dehydrogenase *in vivo*, but not *in vitro*. *In vivo* inhibition by butyraldoxime results in accumulation of acetaldehyde when mice are subsequently treated with alcohol[61].

Butyraldoxime is unusual among aldehyde dehydrogenase inhibitors

in that it markedly inhibits hepatic alcohol dehydrogenase[61]. This action of butyraldoxime is not unexpected in view of the earlier observation that hydroxylamine and pyruvoxime inhibit alcohol dehydrogenase *in vitro*[65]. With a short interval between administration of butyraldoxime and alcohol, acetaldehyde accumulation is suppressed and alcohol disappearance is retarded[61], because of the concomitant inhibition of the rate-limiting alcohol dehydrogenase step.

Other Aldehyde Dehydrogenase Inhibitors - In addition to the four types of compounds discussed above, a substantial number of other substances elicit a disulfiram-like interaction with alcohol: tolazoline, Win 13,099 (N,N'-1,4-xylylenebis-[N-ethyldichloroacetamide]), Win 18,466 (N,N'-octamethylenebis-[dichloroacetamide]), phenformin, nitrofuran antimicrobial agents, antipyrine, aminopyrine, phenylbutazone, animal charcoal, *Coprinus atramentarius* mushroom, etc. Presumably, all of these substances may provoke such a reaction by blocking hepatic aldehyde dehydrogenase, with resultant accumulation of acetaldehyde after consumption of alcohol. A detailed discussion of these inhibitors is not possible here; pertinent references are available[2,54,59,66,67]. Although disulfiram-like activity has usually been discovered as a side-effect of chronic exposure to drugs or chemicals, one systematic attempt to find drugs with such activity in human volunteers has been made[68]. Out of seventy-one miscellaneous drugs tested, only one (tolazoline) yielded the sought interaction with alcohol. A search for new aldehyde dehydrogenase inhibitors can more efficiently be accomplished by *in vivo* biochemical methods in small animals, such as ascertaining drug effects on hepatic enzyme activity, or determining whether acetaldehyde accumulates after a dose of alcohol. An even simpler, though indirect screening method is potentially available, by the use of fluoroacetaldehyde toxicity (cf.[69]), analogously to the use of m-fluorophenylalanine toxicity in uncovering inhibitors of phenylalanine hydroxylase[70].

Metronidazole

By reporting that "prolonged treatment with metronidazole (Flagyl) decreases the compulsive drive for liquor", Taylor[71] instigated a flurry of research and publications on this drug, the end of which is not yet in sight. Only a few of the patients to whom Taylor administered metronidazole (a well known nitroimidazole derivative with trichomonacidal activity) were alcoholics, but these volunteered that their subjective response to alcohol was altered. It is of more than passing interest that in the initial case history described in detail, the patient also suffered from diabetes mellitus which was "well controlled with tolbutamide".

Nevertheless, further study led Taylor[71] to the belief that metronidazole exhibits a multiplicity of actions in alcoholics: improvement in delirium tremens, altered peripheral and central responses to alcohol, decreased psychological drive for and subsequent aversion to alcohol, and a disulfiram-like response when metronidazole and alcohol were con-

sumed concomitantly. Some of these actions are perplexing in a drug which had been widely used well prior to Taylor's disclosure without reports of anti-alcohol effects.

Equally perplexing is the failure of subsequent directed studies, taken as a whole, to illuminate Taylor's assertions. As a deterrent in chronic alcoholism, for example, metronidazole's activity was endorsed by Lehmann et al.[72-74], by Semer et al.[75,76], and by several Italian and Scandinavian workers[77-84]. Yet other clinicians have been unable to confirm this effect[85-90] and there is little basis on which to choose between the positive and negative results. One report[91] has adopted a middle ground: While overall data from this substantial double blind study were negative, some patients were felt to exhibit a "'metronidazole effect' (alcohol tasted different, aversion to alcohol, development of inebriate status on much less alcohol than when untreated)". The authors nevertheless concluded that "if metronidazole has a place in the management of alcoholism, it is a relatively minor one".

One might assume in any case that the question of whether metronidazole plus alcohol leads to acetaldehyde accumulation and the acetaldehyde syndrome could be confirmed unequivocally. But even this remains unresolved. Although several clinicians report clinical evidence of acetaldehyde accumulation, others are as emphatic in denying that it occurs. Disparate evidence on the effect of metronidazole on alcohol levels in man also exists: Rowe and Atkinson[92] saw no effect on the rate of blood alcohol disappearance using themselves as subjects, while Montanini et al.[93] reported decreased disappearance of blood alcohol after metronidazole.

Studies with alcohol and aldehyde dehydrogenases have not been enlightening. Two properties of metronidazole complicate the interpretation of experiments with these liver, NAD^+-dependent enzymes: (a) The strong absorption peak of metronidazole itself at 325 mμ obscures changes at the 340 mμ maximum of NADH[94,95], and (b) the nonenzymatic interaction of metronidazole with NADH, causing disappearance of the 340 mμ peak, could result in inhibition more apparent than real in customary assays dependent on absorbance measurements at this wavelength[95]. Dietrich[21] reported *in vitro* but not *in vivo* inhibition of indoleacetaldehyde oxidation in rabbits by metronidazole. Inhibition of animal or human liver alcohol dehydrogenase *in vitro* has been seen, but only at high concentrations of drug ($\sim 10^{-3}$M)[94,96,97]. Both the normal and atypical forms of human alcohol dehydrogenase were blocked to the same extent[98].

At least one mechanism other than inhibition of aldehyde dehydrogenase has been advanced to explain the anti-alcohol effect of metronidazole. Kissen and Gross[7] and Gelder and Edwards[89], although unconvinced of a useful therapeutic effect of metronidazole in alcoholics, nevertheless noted that metronidazole alters the taste of alcohol adversely; previous publications, when examined retrospectively, offer support for this suggestion.

Regardless of mechanism, one might expect that if metronidazole makes alcohol aversive in man, comparable activity would be detectable in laboratory animals. In the only study directed toward this point, such an effect has in fact been reported. Rats which consumed alcohol either because of genetic factors, or because of prolonged forced consumption, significantly decreased their alcohol consumption after metronidazole[99].

Miscellaneous Drugs

NAD$^+$ (DPN) - In an extraordinary claim, O'Hollaren[100] reported that the coenzyme, nicotinamide adenine dinucleotide (NAD$^+$), "greatly reduces, and in some cases completely removes the craving for alcohol which is responsible for alcohol addiction...". Even more extravagant are O'Hollaren's claims[101] for the use of NAD$^+$ in drug addiction in general ("complete, immediate, total and permanent withdrawal....Addictions treated...include: heroin, opium, morphine, meperidine, codeine, alcohol, methadone, cocaine, amphetamines, barbiturates, and tranquilizers. Craving for the addictive drug, even heroin and opium, is completely removed."). Needless to say, published confirmation of these claims has not appeared. It should nevertheless be noted in passing that nadide has been adopted as the nonproprietary name (trademark: Enzopride) for NAD$^+$, with the therapeutic claim of "treatment of alcoholism and drug addiction"[102].

The rationale for O'Hollaren's alcohol studies[100] is that NAD$^+$ is capable of "rapidly removing...toxic metabolites [of alcohol] from the nervous system". This rationale is based on the finding by Beer and Quastel[103] that NAD$^+$ reverses inhibition exerted by acetaldehyde on the respiration of rat brain mitochondria, probably by facilitating acetaldehyde metabolism. (Brain aldehyde dehydrogenase is contained in mitochondria[104].) It is well known that the metabolism of administered alcohol in animals is accompanied by a decrease in hepatic NAD$^+$/NADH ratio in the whole cell[105] and in the cytoplasmic and mitochondrial compartments[106]. Other tissues, such as heart and kidney, also show decreased NAD$^+$/NADH ratio after alcohol, probably because of the concomitant increase in circulating levels of lactate or other reducing metabolites[105]. This lowering of the hepatic NAD$^+$/NADH ratio is believed to cause a number of alcohol-induced "derangements" in intermediary metabolism[107]. Two other consequences of a reduction in the NAD$^+$/NADH ratio include a probable decrease in rates of alcohol and acetaldehyde oxidation (cf. section on antidiabetic sulfonylureas) and a shift in the peripheral metabolism of biogenic monoamines from acidic to neutral metabolites.

How the decrease in NAD$^+$/NADH ratio after alcohol may be related to the development of alcoholism itself is unknown. A means of increasing this ratio could facilitate removal of alcohol or acetaldehyde from the body, thereby possibly preventing development of addiction, because of insufficient buildup of either substance. It is notable, in view of O'Hollaren's claims for NAD$^+$, that neither nicotinamide nor

NAD^+ itself has an effect on the rate of disappearance of alcohol or acetaldehyde in rats[108]. Other compounds that can serve *in vivo* as a source of NAD^+, such as pyruvate[109] and fructose[110], or that can maintain a normal $NAD^+/NADH$ ratio in alcohol-treated rats, such as promethazine and chlorcyclizine[111], have been reported to enhance the rate of disappearance of alcohol from blood.

Drugs reported to alter the taste of alcohol - As noted above, metronidazole has been reported to alter the taste for alcohol. A more drastic approach to making alcohol taste aversive has been reported by Haumonté[112]. Potassium thiocyanate (Rhocya; rhodanate of potassium) at doses which "vary between 60 and 100 drops a day" serves this purpose, although "toxicity is not negligible", leading Haumonté to confine its use "to old, feeble or weak willed patients"[113]! Older studies on other incompatabilities of KSCN and alcohol have been reviewed[66].

An extract from *Adiantum capillus-veneris*, purportedly containing flavaspidic acid as the active principle, has also been reported to cause aversion to the taste of alcohol in man[114].

Drugs reported to decrease alcohol preference in rodents - In addition to metronidazole[99], disulfiram[59,115], sulfonylureas[59], and butyraldoxime[61], several other drugs have been found to reduce alcohol preference in rodents exposed to alcohol vs. water choice protocols. These include glutamine, several disulfiram congeners, amphetamine, alloxan, and estrogens. Readers interested in primary sources, or in the methodological intricacies and pitfalls of such studies, can consult reviews[1,116].

Many recent papers describe the ability of alcohol to shift the metabolism of serotonin *in vitro*[117,118] and *in vivo*[119-121] from the normal 5HIAA end product to the neutrals, 5-hydroxyindoleacetaldehyde and 5-hydroxytryptophol. This diversion occurs mainly in peripheral metabolism; whether or not it also occurs in the central nervous system, and whether the excess neutral metabolites formed peripherally can enter brain are questions currently being debated[118,121-123]. Of course, an analogous alteration in the metabolism of norepinephrine can also occur as a result of alcohol treatment[124]. These alcohol-induced changes could result from the altered availability of NAD^+ and NADH arising from alcohol catabolism, or from competitive inhibition of aldehyde dehydrogenase by acetaldehyde[67,119,124,125]. The observation by Myers and Veale[126] that p-chlorophenylalanine, which selectively depletes serotonin in brain by blocking tryptophan hydroxylation[127], strongly decreases alcohol preference in Long-Evans rats, even after drug treatment is discontinued, is therefore of considerable interest, and raises new speculation about a possible relationship between alcoholism and serotonin metabolism. Perhaps bearing on such speculation is a recent finding that p-chlorophenylalanine can antagonize the development of tolerance and dependence towards morphine in mice[128].

Alcohol selection in rats is also slightly decreased by α-methyl-

tyrosine, an inhibitor of norepinephrine biosynthesis, but after discontinuation of this inhibitor, alcohol preference rapidly returns to basal levels[126].

General Comment - It is evident from the above that recent years have witnessed few serious new medicinal chemical approaches to the deterrence of alcohol consumption. The apparent dearth of interest in this area is corroborated by the small number of pharmaceutically-oriented researchers in attendance at the 28th International Congress on Alcohol and Alcoholism, held in Washington in Sept., 1968. Perhaps the initiation, within NIMH, of a federally-sponsored National Center for the Prevention and Control of Alcoholism[129] (part of its research program has been described[130]) will spur greater attention to this major problem. It is, after all, ironic that experimental psychopharmacology, which relies so heavily on drug-induced models of aberrant behavior, should not develop new rationales and methodology to deal with an important medical disorder itself characterized by chronic, excess consumption of a specific drug.

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Chapter 23. Drug Metabolism

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Introduction - The interest in drug metabolism has steadily increased during the last several years. In addition to routine studies of absorption, excretion, and the metabolic fate of individual drugs, more attention has been given to drug interactions and to various environmental factors affecting drug metabolism.

There will be no attempt in this communication to review all papers on drug metabolism published during 1968. It will be rather an arbitrary selection of recent papers which will provide examples of research activities in the area of drug metabolism. Hopefully, this information will be of value to the medicinal chemist and will stimulate his interest in drug metabolism. For a more comprehensive treatment of new developments in drug metabolism, the reader is referred to recent publications[1,2] and review articles [3,4].

Factors Affecting Drug Metabolism

Genetic and Environmental - Species differences in response to drugs have been of great concern in drug evaluation. Large variations in rates and patterns of drug metabolism between species are known to exist. They may be due in part to differences in renal excretion of drugs, their binding to plasma proteins, and to differences in the amounts of drug metabolizing enzymes. Many studies suggest that the underlying causes of these differences are genetic in origin. For example, Vesell and Page[5] concluded that the large variations in phenylbutazone metabolism which exist among individuals seem to be genetically determined. White and Price Evans[6] observed that individuals can be phenotyped as slow or rapid acetylators and that significant variability existed even between individuals of the same phenotype. It has been pointed out[7,8,9] that in interpreting the genetically controlled differences in drug metabolism though, one must also consider environmental factors such as exposure to drugs, insecticides, and environmental chemicals.

The space age requires the consideration of still another environmental factor, namely the effect of altitude on drug metabolism, since it has been shown that mice maintained at 18,000 feet exhibited an increased metabolism of hexobarbital[10]. Dietary proteins are also important factors in drug metabolism. It was shown that feeding rats a high protein diet led to an increase, and feeding a low or non-protein diet, to a decrease in metabolism of drugs by the liver microsomes[11]. Drug

toxicities were also affected by the content of the diet; high protein diets exhibited in most cases lower toxicities[11]. The metabolism and the excretion of some drugs are pH dependent[12,13], and this dependence (pH-sensitivity) for ephedrines in man varied with the methylation of the amine group[12].

Hormonal Effects - Radzialowski and Bousquet[14] observed a circadian rhythm in drug metabolism in the rat and mouse, which was abolished by steroid administration. Their investigation has suggested further that the adrenal gland regulated the rhythm in oxidative metabolism, possibly by affecting enzyme synthesis. The circadian rhythm in reductive metabolism however, was not regulated by the adrenals[14]. In other studies, adrenalectomy reduced the rate of metabolism of ethylmorphine, aniline, and hexobarbital[15]. Hydrocortisone treatment or cold stressing of these rats resulted in an increase in ethylmorphine or aniline metabolism but a further decrease in the rate of hexobarbital metabolism.

The role of the pituitary in drug metabolism was demonstrated by the decreased metabolism of hexobarbital, aminopyrine, and p-nitrobenzoic acid in rats bearing a pituitary mammotropic tumor[16]. It was suggested that the anterior pituitary hormones secreted by the tumor were responsible for the observed decrease in hepatic microsomal metabolism[16]. This finding was supported by the demonstration that somatotropin, corticotropin, and prolactin decreased the metabolism of hexobarbital and aminopyrine in rats[17]. It was also shown that the presence of another tumor, the Walker 256 carcinosarcoma, reduced the hydroxylation of amphetamine in rats[18].

Drug Interactions - Many substances are known to affect drug activity by increasing or reducing the rate of drug metabolism. Such interactions are of particular importance in multiple or continuous drug therapy. For instance, phenobarbital enhances the hydroxylation of warfarin, which is probably responsible for the decreased anticoagulant action of warfarin in man given phenobarbital[8]. Phenobarbital also reduces the biological effectiveness of estrogen by inducing its metabolism[19]. Phenobarbital has also been suggested as a therapeutic agent in the treatment of syndromes associated with overproduction of ovarian steroids[19].

Diphenylhydantoin, used in treatment of epilepsy, demonstrated increased toxicity when given to patients maintained on the antitubercular drug isonicotinic acid hydrazide (INH). The increased toxicity was explained as due to an inhibition by INH of the parahydroxylation of diphenylhydantoin, thus causing increased blood levels of the antiepileptic drug[20]. The combination of morphine with amiphenazole (2,4,diamino-5-phenylthiazole) or with THA (1,2,3,4-tetrahydro-9-aminoacridine) was reported useful for relief of chronic pain with no evidence of tolerance or of an abstinence syndrome upon morphine withdrawal[21]. The reason for this beneficial drug combination could be attributed to the inhibition of the metabolism of morphine-N-oxide (a metabolite of morphine) by THA and amiphenazole.

The role of probenecid in multiple drug therapy was studied by several investigators. It was found that probenecid reduced indomethacin excretion, which was postulated to be due to the secretion of both drugs by the same tubular mechanism[22]. In the case of penicillin, however, reduction in tubular secretion by probenecid is only a minor factor in its enhancement of serum antibiotic levels. The major factor appears to be a significant decrease in the apparent distribution volume of penicillin in the presence of probenecid[23]. Probenecid apparently does not inhibit drug metabolism, having no effect on tolbutamide metabolism in man[24]. In addition to probenecid, phenylbutazone can interfere with renal drug excretion. Thus, phenylbutazone potentiated the hypoglycemic effect of acetohexamide by interfering with renal excretion of the active metabolite hydroxyhexamide[25].

A novel interaction between the antidepressant desipramine and phenylbutazone was reported by Consolo[26] who showed that desipramine interferes with intestinal absorption of phenylbutazone in rats. On the other hand, Hammer et al[27] did not observe any effect of desipramine on the half-life of oxyphenylbutazone in man. Since marked species and strain differences are known to exist in the pharmacological effects of tricyclic antidepressants, an attempt was made to correlate these differences with the species differences in protein binding of desipramine[28]. The results do not suggest a high degree of correlation. It has also been reported that the route of administration of imipramine highly influences its metabolism and the tissue drug concentration, particularly in brain[29].

Chronic drug treatment can lead to the development of drug tolerance. Kato[30] described two types of drug tolerance; the "apparent tolerance" mainly due to the induction of drug metabolizing enzymes, and the "real tolerance" mainly due to the decreased tissue sensitivity to the drug.

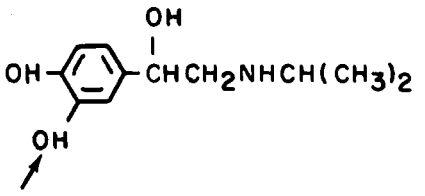
Extrahepatic Metabolism of Drugs - The drug-metabolizing activity of the human placenta was studied by Van Petten et al[31] who showed that the placenta possesses enzymes capable of metabolizing certain drugs by oxidation, deamination, hydroxylation, and hydrolysis with little or no demethylase activity present. Such metabolism of drugs by the placenta may be of major importance to the fetus and should be considered in selecting drug dosages for use during pregnancy[31]. Dixon and Willson[32] reported that phenobarbital pretreatment had no effect on the metabolism of hexobarbital by placental tissue while chlordane stimulated hexobarbital and zoxazolamine metabolism. The finding that environmental chemicals, such as insecticides, could result in increased degradation and inactivation of drugs and various hormones in the fetal circulation is of obvious clinical importance. The authors[32] also pointed out that the placenta supplements the developing fetal liver by metabolizing drugs maximally, early in gestation, when the metabolism by the fetal liver is very limited. Juchau[33] showed differences between human and rodent placental homogenates in their ability to catalyze the reduction of

p-nitrobenzoic acid, and related them to the species difference in tissue flavin content; the reductive reaction could proceed via enzymic and non-enzymic mechanisms. The author also reported that while the reduction of p-nitrobenzoic acid by the hepatic tissue could be stimulated with phenobarbital or 3-methylcholanthrene, the reduction by placental tissue was not affected by these inducing agents[33].

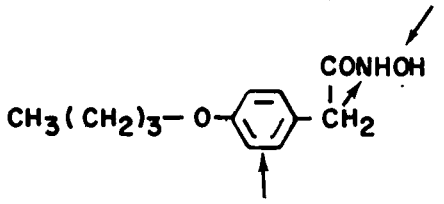
In recent years, the intestinal microflora has been recognized as a site of drug metabolism. Scheline[34] has studied the metabolism of a number of drugs in rat caecal microflora and found extensive metabolic alteration of these compounds.

Metabolic Pathways of Drug Transformation

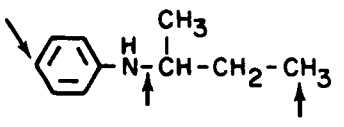
This section, as previously[35], is a digest of metabolic transformations from recent papers, selected to exemplify common and unusual metabolic pathways. The digest includes chemical and/or generic names of the drug, its structural formula, main therapeutic or pharmacological properties, and the species in which studied. The arrows indicate the sites of the described metabolic transformations. The abbreviations used are: UP, unchanged parent compound; (M), major metabolite; (m), minor metabolite; (t), trace amounts; conj., conjugate; gluc., glucuronide conjugate; gly., glycine conjugate; sulf., sulfate conjugate.

1. 

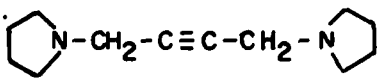
Isoproterenol[36], bronchodilator. Dog. O-methylation; sulf. (position unspecified). Transformation related to route of administration; systemic: O-methylation (M); oral: sulf. (bacterial?). UP, normetanephrine, and unidentified metabolites in urine.

2. 

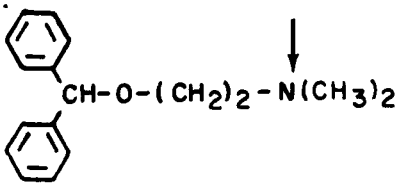
Droxaryl(p-n-butoxyphenylacethydroxamic acid)[37], analgesic. Man. Hydroxylation of phenyl ring (ortho or meta), then gluc. (M) or sulf. (m); reduction and/or hydrolysis in hydroxamic moiety (m). UP in urine following rectal administration higher than after p.o.

3. 

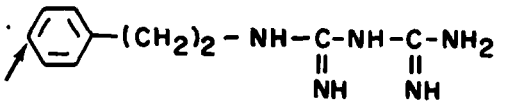
N-sec-Butylaniline[38]. Rat. Hydroxylation of phenyl ring, and dealkylation; 10% of dose in urine as 4-hydroxy-n-sec-butylaniline and 13% as p-amino phenol; side chain oxidation (m) to form 3-phenylaminobutyric acid. No UP in urine. Most of metabolites unidentified. Removal of secondary alkyl substituent from amine.

4. 

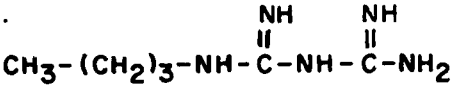
Tremorine[39], cholinergic. Rat. Hydroxylation of pyrrolidine ring (suggested); ring cleavage and oxidation to yield N-(4-pyrrolidino-2-butynyl)-γ-aminobutyric acid; new metabolite, active; ring closure of metabolite to yield oxytremorine (lactam). Lactam formation - represents new enzymatic pathway.

5. 

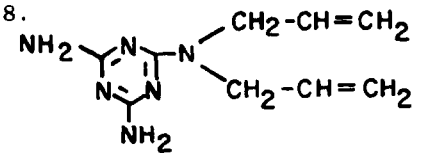
Diphenhydramine[40], antihistamine. Monkey. N-demethylation (m); oxidative deamination to (diphenylmethoxy)acetic acid (m), then glutamine conj. (M). N-oxidation (m); UP (m), unidentified gluc., and benzhydrol (t) also in urine. Previously unrecognized metabolic pathway - glutamine conj. of carboxylic acid.

6. 

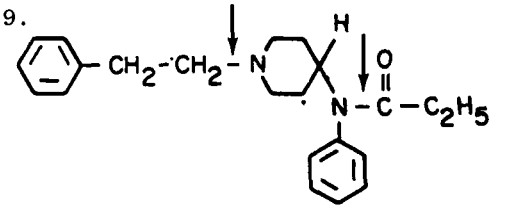
Phenformin (N^1-β-phenethylbiguanide), hypoglycemic. Man, rat. Man[41]: hydroxylation of phenyl ring; Urinary recovery 50% of dose, UP (M). Rat[42]: hydroxylation of phenyl ring (M), then gluc. (M). No UP in urine. Rat in vitro[43]; phenyl hydroxylation by liver blocked by SKF 525-A; split between phenethyl and biguanide moiety suggested.

7. 

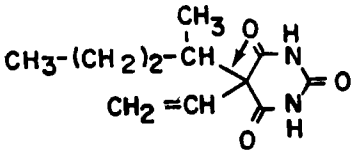
Buformin[41](N^1-n-butylbiguanide), hypoglycemic. Man. UP, 90% of p.o. dose in urine, in contrast to other species which hydroxylate drug.

8. 

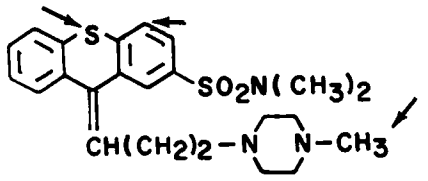
N,N-diallylmelamine[44], vasodilator. Rat, dog, man. Extensively metabolized. N-oxidation (triazine ring, reversible reaction) to form active metabolite, then deallylation and N-reduction (rat); direct deallylation (M) (all species); hydroxylation of allyl group; N-methylation (triazine ring) then deallylation; UP in urine. Species difference - man does not produce active metabolite, (N-hydroxy triazine derivative).

9. 

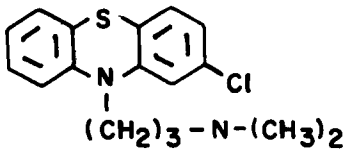
Fentanyl[45], analgesic. Rat. Oxidative dealkylation of phenethyl moiety (M), then N-dealkylation of propionyl side chain (m); hydroxylation of phenethyl (m) and/or dealkylation of propionyl (m). UP and metabolites in urine and feces.

10. 

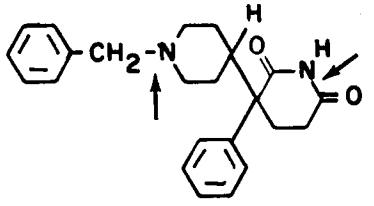
5-(1'-Methyl-n-butyl)-5-vinyl-barbituric acid[46], hypnotic. Man. Dealkylation; 5-methylbarbituric acid and UP in urine.

11. 

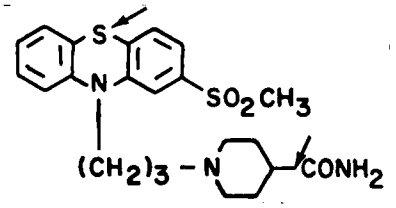
Thiothixene[47], psychotherapeutic. Rat. Extensively metabolized, metabolites in urine, bile, feces. Demethylation of piperazine moiety (M); metabolite in urine; UP (t) in urine and feces.

12. 

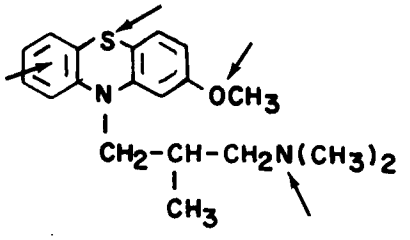
Chlorpromazine[48], tranquilizer. Man. Demethylated metabolites bound to plasma; also in erythrocyte. Chlorophenothiazinyl-propionic acid in plasma.

13. 

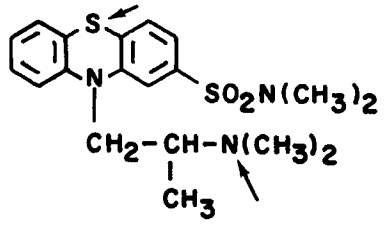
Benzetimide[49], anticholinergic. Rat. Oxidative N-dealkylation (M); ring opening (m). UP and metabolites in urine and feces.

14. 

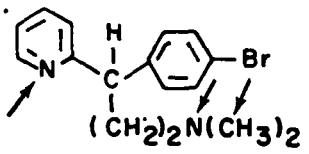
Metopimazine[50], antiemetic. Dog, rabbit. Dog: sulfoxidation, hydroxylation (M), and hydrolysis of carbamoyl moiety; conj. metabolites (gluc.?) and UP in urine. Metabolites in feces. Rabbit: metabolism same as dog; hydrolysis of carbamoyl moiety (M); species difference (quantitative). Low recovery of dose from urine of dog and rabbit; fecal recovery 50% of dose in dog.

15. 

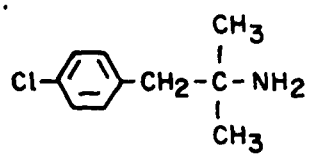
Methotrimeprazine[51], analgesic. Rat, man. Rat: extensively metabolized to sulfoxide and phenolic products. Hydroxylation and/or O-demethylation, metabolites free or gluc. (M) in urine; sulfoxidation then N-demethylation (m) or hydroxylation. UP (1%) in urine of rat and man.

16. 

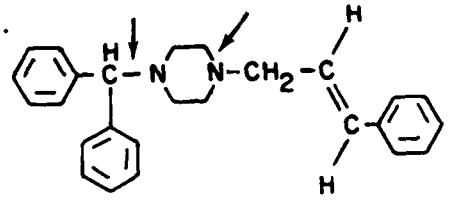
Dimetotiazine[52], sedative. Man and dog. Mono- and di-demethylation of dimethylaminopropyl side chain; sulfoxidation; other unidentified metabolites, hydroxylated and conj.; UP in urine. Low recovery of dose indicated.

17. 

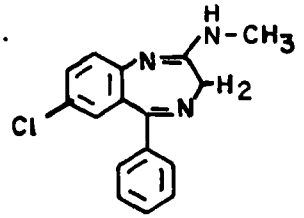
Brompheniramine[53], antihistamine. Dog, man. Mono and di-N-demethylation (M); pyridine N-oxidation (m), dog only; side chain oxidation (m) then gly. (m). UP in urine. Man: UP and demethylated metabolites in equal amounts in urine, no N-oxide detected; Dog: di-demethylated derivative (M). Species difference. Large fraction of urinary metabolites unidentified.

18. 

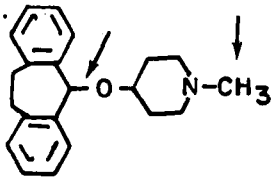
Chlorphentermine[54], anorexigenic. Rat: UP (70-90% of dose) in urine. Mouse (female): Conj. (60% of dose) neither gluc. nor N-acetyl; UP (25% of dose) in urine, unidentified metabolite (m) present. Species difference. Unusual conj. via unrecognized pathway. Non-microsomal metabolism.

19. 

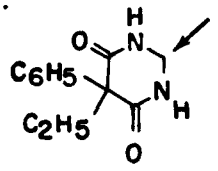
Cinnarizine[55], antihistamine. Rat. Extensively metabolized. N-dealkylation (M), metabolite (benzhydrol) in urine as gluc.; N-oxidation (m). In feces, benzhydrylpiperazine (M), benzophenone and hydroxylated product (M), benzhydrol, and UP.

20. 

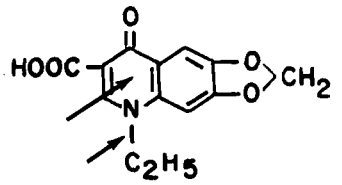
Chlordiazepoxide[56], tranquilizer. Rat. Phenyl ring hydroxylation followed by demethylation (M), and deamination; N-oxide reduction. Metabolites free or conj. Urinary recovery, 30% of dose.

21. 

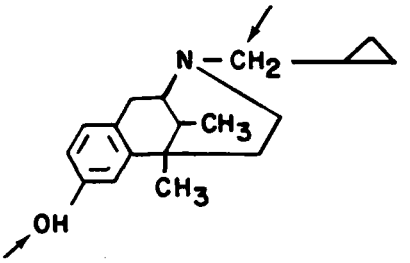
Hepzidine maleate[57], antidepressant. Rat. N-demethylation (M); 50% of the p.o. dose hydrolyzed before absorption; hydrolytic products in urine as conj. (M). UP and metabolites in bile.

22. 

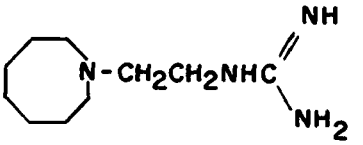
Primidone[58], anticonvulsant. Rabbit. Oxidation and ring opening. Ethylphenylmalondiamide (48% of dose), UP (20% of dose), and phenobarbital (m), in urine. Accumulation of phenobarbital noted upon repeated administration.

23. 

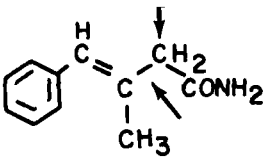
Oxilinic acid[59], antimicrobial. Man. In urine - 8 metabolites. Gluc.(M); oxolinic acid complex (unidentified) (m); N-deethylation. Active metabolites in urine, feces. Other metabolites, unidentified gluc. and non gluc.

24. 

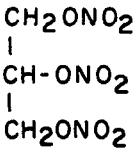
Cyclazocine[60], analgesic. Dog. N-dealkylation, metabolite free and gluc.; UP, free and gluc. (M) in urine; 1 unidentified metabolite. Metabolites in feces. No change in metabolism in tolerant dog. Large portion of dose not accounted for.

25. 

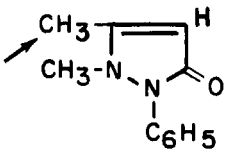
Guanethidine[61], hypotensive. Rat. Single unidentified polar metabolite (M) and UP in urine. Metabolite also in tissue.

26. 

3-Methyl-4-phenyl-3-butenamide[62], hypoglycemic-hypocholesterolemic. Rat, rabbit, man. Hydroxylation of side chain, and phenyl ring; γ-lactone formation and hydroxylation of phenyl ring of lactone. UP in urine.

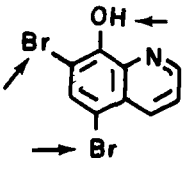
27. 

Glyceryltrinitrate[63], coronary vasodilator. Rat. Denitrated to glycerol then completely metabolized to CO_2; unidentified metabolites in urine. Low recovery (20%) of dose from urine.

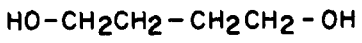
28. 

Antipyrine[64], antipyretic-analgesic. Rat, mouse, guinea pig, rabbit, man. Oxidation of 3-methyl pyrazoline to corresponding alcohol. New metabolite, 3-hydroxymethyl antipyrine analog: Rabbit, guinea pig (m); rat, mouse, (M). Metabolite free in urine.

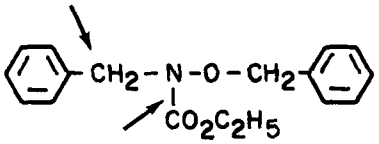
Butylaminoantipyrine[65]. Rabbit, guinea pig, mouse, rat. Dealkylation (M) then acetylation of aminoantipyrine metabolite; hydroxylation (m), related to length of alkyl chain in N-alkyl compound.

29. 

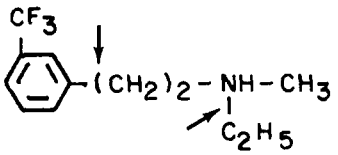
5,7-Dibromo-8-hydroxyquinoline[66], amebicide-bactericide. Man. Gluc. (M); debromination (m); UP (m) in urine. Urinary recovery, 58% of dose.

30. 

1,4-Butanediol[67], CNS depressant. Rat. Oxidation to γ-hydroxybutyric acid. Active metabolite in blood and brain.

31. 

Benzyl-N-benzyl carbethoxyhydroxamate[68], hypolipidemic. Man. Hydroxylation, N-dealkylation, then O-gluc., formation. New metabolite, γ-(benzyloxyamino)benzyl gluc. in urine in addition to known metabolites.

32. 

Fenfluramine[69], anorexigen. Man, dog. Deethylation (m); deethylation and side chain oxidation, then gly. conj. (M); UP (m) and trifluoromethylhippuric acid (M) in urine. Dog, unlike man, free trifluoromethylbenzoic acid in urine. Species difference.

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Chapter 24. Neurotransmitters Revisited

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Introduction - In last year's chapter[1], my late colleague, Dr. Giarman and I attempted to characterize the current status of research on brain neurotransmitter substances. The major topics of that chapter were the basic biochemistry and physiology underlying chemical neurotransmission in the central nervous system (CNS), including a critique of the recent technical advances. Because of the emphasis upon the basics of neurotransmitters, we could not dwell upon the status of information regarding any one neurotransmitter or any one synaptic junction. Therefore, this chapter will deal with the current status of specific central neurotransmitters for particular neuronal junctions.

Let us first recapitulate the primary tenets of the problem. To identify an agonist substance as the actual neurotransmitter for a specifically defined neuronal pathway, we require[2, 3] data showing: 1) that the putative transmitter substance is located in the appropriate nerve endings; 2) that the physiological effect of this transmitter is identical to the effect produced by stimulation of the nerve pathway under investigation; 3) that those drugs which alter the response of the post-synaptic cell to the nerve tract stimulation also alter the response to the agonist substance in an identical manner; 4) that when the pathway is stimulated, the agonist substance is released by the nerve terminals. This last criterion has still to be satisfied for any pathway, and no available technique appears capable of detecting neuronal release at the cellular level[4]. Of the other three criteria, the first requires either micromethods of biochemical analysis or combined cytochemical, histochemical and fine structural analyses. The second and third criteria require electrophysiologic and pharmacologic information, and the selective accessibility of a nerve pathway in order to assess the effects of induced stimulation. The techniques by which such data are obtained were reviewed in detail in last year's chapter.

Acetylcholine (ACh) Mediated Junctions - For many years, ACh has been a commonly accepted neurotransmitter substance[5]. This view is most fully established for the peripheral parasympathetic nervous system[6], for autonomic ganglionic transmission[7], and for the neuromuscular junction[8]. In regards to the CNS, it is widely although not universally accepted, that the so called Renshaw discharge is caused by the release of ACh from the collaterals of spinal motoneurons. When microelectrodes are used to

record from the ventro-lateral portions of the spinal cord, cells are encountered which have rapid frequencies of discharge during the interval after antidromic stimulation of the ventral root. This high frequency rapid discharge is known as the Renshaw discharge and the cell from which the discharge arises has therefore been termed the Renshaw cell[9]. The Renshaw cell, in turn, causes inhibition of the activity of motoneurons and thus represents a negative feedback circuit within the spinal cord. This system has been intensively studied by Eccles and his coworkers and has recently been summarized[10]. The pharmacological data which establishes this pathway (from motoneuron axon collateral to Renshaw cell) as being cholinergic consist of the following observations. After cholinesterase inhibitors or cholinolytic drugs were injected either intravenously or intra-arterially close to the region of the spinal cord from which recordings were being made, the effects of antidromic stimulation were either prolonged or inhibited respectively[11]. The same types of drugs were administered in subsequent studies by the method of microelectrophoresis[12]. These results show that Renshaw activity can be evoked either by antidromic stimulation of the ventral root or by the iontophoresis of ACh, nicotine, or carbachol. The repetitive discharge is also greatly prolonged by the administration of cholinesterase inhibitors such as eserine or neostigmine. The response to ACh and the repetitive discharge are both greatly reduced by the application of a nicotinic blocker, dihydro-beta-erythroidine[13]. All these data thus appear to satisfy the electrophysiologic and pharmacologic criteria for a cholinergic synapse. However, there is disagreement over the histologic existence of the Renshaw cell identified by electrophysiology. Neuroanatomic studies have not demonstrated any cells in this region of the spinal cord which receive axon collaterals from motoneurons and simultaneously give synaptic boutons to motoneurons[14]. However, in the region of the spinal cord from which the Renshaw cell discharge has been recorded, small interneurons can be seen by a variety of cellular staining techniques in which the stain is administered through the recording electrode[15, 16]. Regardless of the exact morphological components of the Renshaw discharge, and despite the great deal of attention devoted to the electrophysiologic phenomena of Renshaw cells, the relevance of the Renshaw pathway to the regulation of spinal cord motoneuron activity in the intact animal has yet to be established[17].

Acetylcholine Receptors - In the many other sites within the CNS at which responsiveness to ACh has been characterized by microelectrophoresis of cholinolytics or cholinesterase inhibitors, the results fail to reveal the nicotinic receptor properties regularly attributed to the Renshaw cell circuit. Instead, the cells exhibit properties which are wholly muscarinic or mixed nicotinic and muscarinic[18]. On the basis of available data, any conclusion that either nicotinic or muscarinic receptor properties are more highly characteristic of the ACh receptor of the CNS is premature. In fact, whether this form of characterization can even be legitimately extrapolated to the brain remains to be shown.

Possible Cholinergic Junctions - There are at least four other areas within the CNS at which the electrophysiologic and pharmacologic analysis of

responses to ACh have been characterized, although not as fully as the Renshaw cell circuit of the spinal cord.

Caudate Nucleus - In this region, noted for high levels of cholinesterase and choline acetyltransferase, a high proportion of spontaneously active cells were found to be activated by ACh[19] and by stimulation of the ventral anterior thalamus[20]. This effect is quite sensitive to the type of anesthesia or surgical preparation of the animal[19]. The effects of the thalamic stimulation and of the ACh application can both be prevented by the local application of atropine[20]. However, in these latter studies, only a small proportion of the total cells tested could be found to be responsive to ACh. While this may have been due to the type of surgical preparation, the data, as presently reported, do not explain how such a small proportion of the neurons found in this area could account for the great biochemical predominance of the cholinergic system.

Thalamus - Cells facilitated by the local application of ACh have been described for two areas of the thalamus: the ventral basal thalamic nuclei[21] and the lateral geniculate nucleus[22]. The responses to ACh were potentiated by cholinesterase inhibitors, and could be blocked with atropine. However, in the ventral basal thalamus, the responses to ACh could also be prevented by dihydro-beta-erythroidine. In an attempt to locate the possible cholinergic tracts, ACh blockers were evaluated for effects upon stimulation of the major thalamic afferents (visual inputs to the lateral geniculate and cutaneous sensory inputs to the ventral basal thalamus); since cholinolytics had little effect on the response to synaptic stimulation, these sensory pathways are probably not the cholinergic fibers. Thus, the electrophysiology and pharmacology are compatible with the existence of ACh as a transmitter for a pathway which was anatomically uncharacterized. Thalamic cells activated by stimulation of the brachium conjunctivum (containing cerebellar efferent axons) are also facilitated by ACh, although cholinolytics do not block the synaptic activation[23].

Cortex - ACh-sensitive cells have also been described for the cerebral cortex, particularly the visual[24] and somato-sensory areas[25], and for cerebellar[26, 27] and limbic[28] cortex. In the visual cortex, the effects of light flashes could be potentiated by application of cholinesterase inhibitors. However, in none of the cortical areas, can the precise pathway by which such presumptive ACh-containing neurons arises and distributes itself, be determined with any accuracy.

Location of ACh Mediated Pathways - Each of these pharmacologic studies would be strengthened by data on the histology of ACh-containing nerve tracts. In a recent symposium devoted to "Central Cholinergic Transmission"[29] it was concluded that histochemical staining for acetylcholinesterase was of only partial help in determining such presumtive cholinergic pathways. In many cases, the results of this histochemical staining simply do not agree with the results of biochemical or electrophysiologic experiments, a particular case in point being the cerebellar cortex. In

the cerebellum, the distribution of cholinesterase positive nerve fibers in the molecular layer[30] does not completely agree with the concentration of ACh found in isolated mossy fiber terminals[31]. Furthermore, the distribution of both these biochemical entities also disagrees with the sensitivity of Purkinje cells to the microelectrophoresis of ACh[26]. These data suggest that the histochemical use of cholinesterase staining as a clue to the distribution of cholinergic nerve circuits has been frequently over-interpreted[29].

Catecholamine-containing Nerve Junctions - While a high degree of enthusiasm has been associated with brain catecholamine research, progress in understanding the cellular function of these monoamines has been slow. Several early studies[32] suggested that neither norepinephrine (NE) nor dopamine (DM) exerted much effect on the discharge rate of cells tested in the spinal cord, brain stem, and cerebral cortex. However, these results appear to have been falsely negativistic, in that subsequent studies[33], particularly in unanesthetized animals, have indicated a high degree of responsiveness to both catecholamines. The circuitry of the catecholamine-containing neurons has been intensively studied by the fluorescence histochemists, who propose that all such nerves arise from cell bodies contained in the brain stem or pons and that they project diffusely to the entire CNS[34].

Olfactory Bulb - In only one brain region has a potential NE synapse been studied to relative completeness by electrophysiologic and pharmacologic experiments. In the rabbit olfactory bulb, the chief efferent cell projecting to the rhinencephalic cortex is the mitral cell. The mitral cell axons leaving each olfactory bulb form a large myelinated tract known as the lateral olfactory tract. When micro-electrodes are used to record the discharge rate of any olfactory bulb cells, a definite period of inhibition results from stimulation of the lateral olfactory tract[35]. This inhibition is presumably mediated either directly or indirectly by the mitral cells. Intracellular recordings from mitral cells during lateral olfactory tract stimulation demonstrate an inhibitory post-synaptic potential of 50-150 milliseconds[36]. When the mitral cells were tested with potential neurotransmitters, ACH NE, and serotonin (5-HT) were all able to depress spontaneous activity[37]. Thus, all three substances - and presumably others - could have been potential transmitters for the inhibitory circuit from the mitral cell axon collaterals. However, only those substances which interfered with the response to NE, (including dibenamine, and LSD) or substances which would deplete brain stores of NE (such as alpha methyl-m-tyrosine) were able to reduce the duration of the inhibitory period following lateral olfactory tract stimulation[38, 39]. Subsequent study has shown that the olfactory bulb receives NE-fluorescent nerve terminals, particularly in the layer of the olfactory bulb deep to the mitral cells[40]. In addition, slices of olfactory bulb are capable of taking up radioactive NE and releasing it upon electrical stimulation[41]. However, as in each of the previous cases of junctions well-defined by electrophysiology and pharmacology, no precise cytology links the mitral cell axon collaterals and the nerve terminals releasing NE.

Catecholamine Receptors - Cells in the CNS most commonly respond to NE by slowing their rates of discharge[42]. However, a high proportion of neurons in the brain stem increase their discharge rate as the typical response to NE[43]. Chlorpromazine effects may be mediated through interactions with NE-containing cell bodies in these areas because the drug is able to block this facilitatory response to NE[44]. In addition, other drugs effective in blocking response to NE are phenoxybenzamine[45], dichloroisoproteronol[46], and alpha-methyl NE[47].

Catecholamine Receptiveness - In the spinal cord, motoneurons can be depressed by NE[48] while Renshaw cells and other types of interneurons can be either facilitated or depressed by NE[49]. Cells in the lateral geniculate nucleus which project to the visual cortex are facilitated by NE[22], while the other geniculate cells respond by slowing[50]. In the cerebellar cortex of the rat, the response to NE is entirely one of slowing the spontaneous discharge rate[51]. The same qualitative response is seen in 30-50% of the cells tested in the cerebral cortex[52], hippocampus[53], septum[54], and medial geniculate[55]. All of these latter experimental reports must be considered as pharmacologic phenomena until the pathways involved can be selectively stimulated to verify the existence of specific NE-containing junctions.

Dopamine - In the case of DM, the most intensively studied circuit involves those cells arising in the substantia nigra and projecting through the lateral brain stem and internal capsule into the caudate nucleus. This pathway has been proposed on the basis of fluorescence histochemical data[56], and has been partially confirmed by more classical neuroanatomical techniques, including electron microscopy of degenerating caudate nerve endings after substantia nigra lesions[57]. In addition, when repetitive bursts of electrical stimuli are given to the substantia nigra, approximately one-half of the cells within the caudate nucleus exhibit slowing of their discharge rate[58]. The same response is seen with electrophoresis of DM[19, 59]. However, no specific DM blocking agents or DM depleting agents have thus far been applied to this potential pathway. Slowing of caudate cells also occurs with thalamic stimulation, and this connection would not correlate with fluorescence data[60]. In Paralysis Agitans or Parkinson's Disease, the cells of the substantia nigra lose their typical hyperpigmentation while the caudate nucleus contains much lower concentrations of both DM, and 5-HT[61]. Moreover, many of the progressive crippling symptoms and neurological deficits of this disease can be alleviated by the chronic administration of dihydroxyphenylalanine (DOPA), the precursor amino acid for DM[62]. However, when this disease is produced experimentally many of the symptoms appear with lesions interrupting possible 5-HT-containing nerve fibers of the medial brain stem, as well as with destruction of the DM-containing fibers[63]. Thus, in the case of this potential DM-containing pathway, the cellular circuitry is only partially clarified. Pharmacologic data are still required to show that the same drugs will block the stimulation of the nigra and the response to microelectrophoresis of DM. Preliminary data indicates that stimulation of this pathway will result in the release of homovanillic acid (a dopamine catabolite) into the lateral ventrical,

opposite the caudate[64]. Since the caudate nucleus contains high levels of DM, 5-HT, ACh, and cholinesterase, this area would seem to be ideal for assessing the specificity of the various morphological techniques used in location of neurotransmitters. Such a study has yet to be reported.

Serotonin - As in the case of the catecholamines, the number of review articles on possible cellular effects of 5-HT in the brain closely approaches the number of pages of original data describing such effects. Neurons responding to microelectrophoresis of 5-HT have been reported for virtually every portion of the CNS, including cerebral cortex, cerebellar cortex, spinal cord, brain stem, lateral geniculate nucleus, hypothalamus and septum. The most commonly reported 5-HT effect is slowing of spontaneous discharge rate[65]. However, in certain areas (brain stem, cerebral cortex, lateral geniculate nucleus and spinal cord) cells which increase their discharge rate in response to 5-HT have also been observed[42]. Furthermore, in contrast to the depressant 5-HT responses which do not appear to have selective specific blocking agents, the facilitatory responses to 5-HT have been blocked by LSD, analogues of LSD and barbituates[66].

The 5-HT-containing cell bodies are located by fluorescence histochemistry in brain stem and pontine nuclei, and project diffusely to the entire CNS. Large lesions of the brain stem are capable of eliminating almost all brain 5-HT[67]. The clustering of these cells in the midline of the brain stem has made them accessible to stimulation experiments on behavioral and biochemical signs of induced neuronal hyperactivity[68]. Nevertheless, despite the histologic and the electron microscopic evidence connecting certain of the 5-HT-containing cellular areas to various portions of the CNS, no study combining the effects of stimulation of these pathways, of responsiveness to 5-HT, and of 5-HT blocking agents has yet been combined to produce a complete set of data on the existence of specific 5-HT-mediated pathways.

Recent data suggest a 5-HT-pathway may project from the lateral reticular formation to the midline of the pons[69]. It is of interest that LSD appears to be capable of blocking the response of neurons to both NE and to 5-HT, and that microelectrophoresis of both these substances appear to increase the firing rate of the 5-HT-containing nerve cells. These observations coincide neatly with previous reports that intravenous LSD slows cells presumed to contain 5-HT[70] and that metabolic turnover of 5-HT is also slowed by LSD[71]. Of course, none of these experiments are able to explain the mechanism by which LSD produces profound distortion of sensory preception[72].

Amino Acids - The naturally occurring amino acids, gamma-aminobutyric acid (GABA), glutamate and glycine have sparked much controversy concerning their potential function as neurotransmitter roles. Here, there can be no doubt that these molecules are highly effective agents capable of either acutely depressing (in the case of glycine and GABA) or facilitating discharge rate (in the case of glutamate)[73, 74]. The glutamate effect shows

depolarization while the depressant effects are accompanied by hyperpolarizing transmembrane potential changes identical to those produced by natural inhibitory nerve pathways[75]. In spite of these highly sophisticated comparisons of the effects of the amino acids and naturally occurring synaptic transmitters, there is still great reluctance in accepting the amino acids as neurotransmitters. The amino acids appear to occur almost universally throughout the brain, without the degree of regional variation found for ACh and the monoamines. Only recently has active accumulation of GABA by certain types of nerve endings been demonstrated[76], although the enzymes for its synthesis have long been associated with the nerve ending fraction of brain homogenates[77]. Almost all cells in the brain which have been tested show responsiveness to both excitatory and to inhibitory amino acids, a point which, in the eyes of some, further diminishes their chances of being specific neurotransmitters. Since the substances are closely related to naturally occurring intermediates in carbohydrate metabolism, data is needed to clarify the mechanisms by which neurons might prevent such active substances from affecting their own discharge rate as they are normally synthesized. A highly structured mechanism must be sought that can prevent naturally occurring substrates from giving false transmission signals and still permit the same molecules to act as "true transmitters" at presumably near-by synaptic sites.

While no specific synaptic junction has been proposed as glutamate mediated, two potential junctions have been related to the inhibitory amino acids. In the cerebral cortex, GABA has been proposed as the transmitter mediating the prolonged type of inhibition caused by surface stimulation of the cortex[75]. This interpretation is based almost entirely upon electrophysiological evidence relating the equilibrium potential for the naturally occurring synaptic transmitter to the equilibrium potential resulting from microelectrophoresis of GABA during simultaneous intracellular recording and extracellular drug application. Those blocking agents tried resulted in compatible negative evidence, in that no drugs eliminated the response to GABA. Gross release of GABA from the superfused cerebral cortex has also been reported[78]. However, no evidence relating the GABA concentration of the cortex to specific neurons has yet been possible due to lack of specific histochemical reactions for this substance. The inhibitory effect of cerebellar Purkinje cells on brain stem neurons may also be mediated by GABA[79].

In the spinal cord, glycine has been proposed as an inhibitory neurotransmitter for motoneurons since its microelectrophoresis can result in the production of a hyperpolarizing change whose equilibrium potential is close to that for naturally occurring inhibitory paths ending on the motoneuron[80]. In addition, the glycine concentration of the spinal cord diminished by anoxic destruction of interneurons[81]. Furthermore, strychnine, a drug which reduces the inhibitory potentials produced by synaptic stimulation of motoneurons has been shown to block the response to glycine without changing the response to GABA[82, 83]. Location of radioactive glycine by autoradiography is confused by the possibility that the glycine is taken up and incorporated into protein[84]; thus, this evidence cannot be used as an exclusive method for locating possible glycine-storing and transmitting interneurons. Some such cytological evidence is needed to verify

the existence of nerve cells transmitting by specific amino acids.

Other Possible Neurotransmitters - Even if the synapses for which only partial data is available were eventually proven to be mediated by one or more of the substances described above, there would still be a multitude of synaptic connections for which there are no clues to identify the transmitter substance. Mainly, this situation arises from the fact that possible transmitters cannot be located within single nerve endings even if we knew what substance to try to identify. Conceivably, sophisticated analytical tools such as the mass spectrometer will someday be combined with histological techniques to permit this type of analysis to take place. Until this happens, one is more or less forced to seek naturally occurring substances, with neuronal effects in other organs, for possible effectiveness as brain transmitters. In this connection, we shall briefly consider two substances whose possible effects as brain neurotransmitters stem from their discovery in non-neuronal tissues, namely histamine and prostaglandin. Both substances have been shown to occur within the CNS[85] and their gross release from the cerebral[86] and cerebellar[87] cortex has been described. While histamine has been associated with the nerve ending fractions of cortical and hypothalamic homogenates[88], there has been no definite association of the prostaglandins with nerve fibers nor has there been any conclusive work associating either of these substances to particular pathways. Microelectrophoresis of histamine in the cerebral cortex results mainly in depression of spontaneous activity[89], while little direct effect with any prostaglandin has been reported[90].

Functional Roles for Neurotransmitters - Statements are often made to the effect that "Norepinephrine seems to be an excitatory substance for the brain" or "5-HT causes central depression". Such statements usually refer to data obtained by observing the behavior of the whole animal in some type of psychological measurement in response to drugs which when given parenterally affect generalized storage or synthesis of these transmitter substances. While this type of data, if obtained in an interpretable manner, would be extremely valuable in postulating the possible mechanism by which these brain substances might causally interact in states of mental illness, such sweeping generalizations do not appear to have a factual basis. The data reviewed above describe the rudimentary states of knowledge for a minute proportion of the central synaptic connections. Even in those very few cases in which there is relative completeness of data, the purpose of such transmitters in the functioning of, for example, the olfactory bulb or, the spinal cord, remains in doubt. At the opposite pole, there is relatively complete circumstantial data suggesting that both NE, and 5-HT, as well as ACh, have integral but uncertain roles in the mechanisms responsible for the onset, duration, and termination of sleep[91]. Similarly, those brain areas integrating the information responsible for neurosecretion also appear to have intimate association with the nerve fibers containing NE and 5-HT[92]. However, there is a wide "synapse gap" between the knowledge obtained at the cellular level and, the integration of this knowledge into behavioral functions and the relation of these monoamines to those particular functions. One may ask with good reason, is it

fair to speak of "brain NE" or "brain 5-HT"? Clearly, the cells containing these substances arise in many different parts of the brain stem and the overlap or specificity of their connections and of their own afferent pathways is largely unknown. Until such information becomes available it would seem most prudent to think that "the norepinephrine" may serve various functions in different portions of the CNS. Information at the cellular level will be essential in determining the role of these substances for individual brain functions.

Although the quest for better understanding of the role of neurotransmitters at the cellular level is far from over, an even greater challenge is the need for research techniques to correlate groups of cells functionally and to relate the activity of these groups of cells to the so-called higher integrative capacities of the brain.

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Section VI - Topics in Chemistry

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Chapter 25. Steroids

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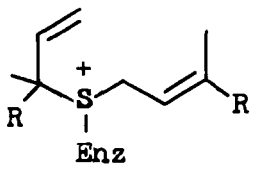
Introduction - Valuable contributions continue to be made in unfolding the intimate mechanisms of sterol biosynthesis.[1] While the steroid molecule continues to be a useful model for organic synthesis, structural modification of its nucleus has not yielded mammalian hormones with novel or markedly enhanced biological properties. The discovery of new hormones and biologically active substances in the plant and lower animal kingdom has sustained the chemist's interest in steroids. As the chemist adds to the understanding of the role of the steroid hormone in the life process, the biochemist quickens his search for the mechanism of its action.[2]

Biosynthesis of Sterols - On the basis that enzymatic coupling of two farnesyl pyrophosphate units to yield squalene involves a thio derivative of the type 1, models have been prepared,[3,4] converted to the corresponding sulfonium ylids, and rearranged easily to squalene-like hydrocarbons.



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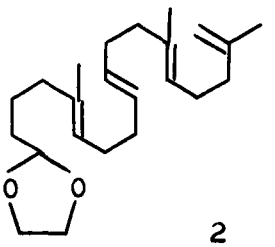

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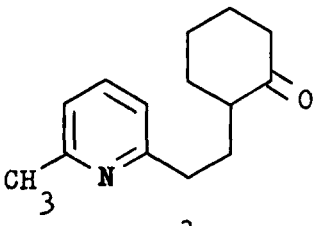
Involvement of a cyclopropyl type intermediate in the coupling reaction appears to be ruled out.[5] The biological significance of epoxysqualene in sterol biosynthesis has been affirmed by its conversion to fusidic acid[6], yeast sterols,[7] and by the annelation of modified squalene oxide precursors.[1,8] In the transformation of lanosterol to cholesterol, evidence has accumulated that the removal of the C-14 methyl group involves the loss of the 15α hydrogen atom[9,10] and that, contrary to earlier conclusions, the loss of the 4α and not the 4β-methyl occurs first.[11] The configuration of the hydrogens gained or lost at carbon atoms 5 to 9 in the migration of the double bond at the C-8 to the C-5 position in the conversion of lanosterol to cholesterol has been the subject of several communications.[9,12,13] The stereochemistry of the hydrogen atoms involved in the introduction of the C-24 alkyl

groups in phytosterols has also been investigated.[13,15]

Total and Partial Synthesis - Recent developments in the total and partial synthesis of steroidal estrogens and heterocycles have been reviewed.[16,17] A novel total synthesis has been based on a nonenzymatic, biogenetic-like, polyolefinic cyclization as the key step, namely, the conversion of the ketal 2 in a stereoselective manner to a tetracyclic compound.[18]

In addition to the biogenetic type synthesis, several interesting approaches to the construction of carbocyclic systems have been developed. The successful use of 6-vinyl-2-picoline as a bisannelating agent in the preparation of the tricyclic dienone 4 from cyclohexanone has been reported.[19] The synthesis involves a reaction of the pyrrolidine enamine of cyclohexanone with vinyl picoline to yield 3 which, as its ketal, is transformed by Birch reduction and treatment with acid and then base to 4.



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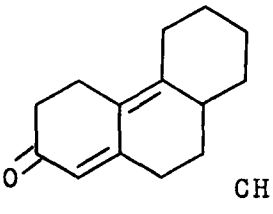

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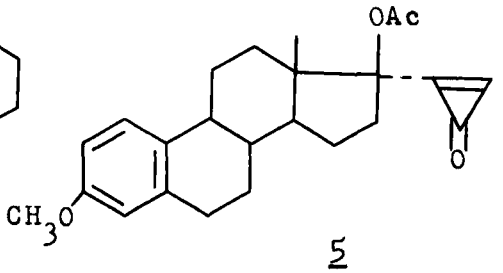

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An approach to the synthesis of 16-ketosteroids utilizes monoalkylation of a 1-tetralone with 2,3-dichloropropene in the presence of methylmagnesium carbonate in DMF.[20] Alkylidenetriphenylphosphoranes and dialkyl phosphonate anions attack a variety of cyclic enol lactones to give intermediate ketophosphoranes which yield, in one step, the desired cyclic α,β-unsaturated ketones.[21] Thus, the reaction of 17β-acetoxy-4-oxaandrost-5-en-3-one with methylene triphenylphosphorane for 18 hours at 20° affords testosterone acetate.

General Reactions - Studies on the addition of halocarbenes to unsaturated steroids continue. Difluorocarbene reacts with the 17-acetylenic side chain of the 17-acetate of ethinyl estradiol 3-methyl ether to yield a cyclopropene derivative which upon acid hydrolysis gives the cyclopropenone 5.[22] The use of phenyl (trihalogeno) mercury precursors results in addition of halogenocarbenes to highly hindered double bonds of steroids.[23] In a typical experiment cholest-7-en-3β-yl benzoate and the chloromercurial react to give a 7α,8α-adduct in 50% yield.

Eniminium salts have been shown to be efficient protecting groups for the Δ^4-3-keto moiety in the synthesis of steroids oxygenated at C-17 and C-21. Thus the pyrrolidinium perchlorate formed from progesterone yields a 17-enol acetate

which by oxidation is transformed to 17-hydroxyprogesterone in good yield.[24] On the other hand, the free bases such as the enamines of cholest-4-en-3-one, testosterone, and androst-4-ene-3,17-dione give good yields of 6-keto derivatives upon autoxidation with air in the presence of ferric or cupric ion.[25]

Oxyanions generated in situ have been shown to be useful intermediates in steroidal synthesis. A method for ethinylation of a 17-ketosteroid followed by concomitant acylation of the intermediate oxyanion to yield a 17β-acetoxy-17α-alkinyl steroid, in a single step, has been reported[26]; also, base treatment of $\Delta^{4,6}$-3-keto steroids in dimethyl sulfoxide followed by acidification with aqueous acid has led to the formation of free enols which can be converted to 3-acetoxy-3,5,7-trienes with acetic anhydride or to 3-keto-5,7-dienes with additional acid.[27]

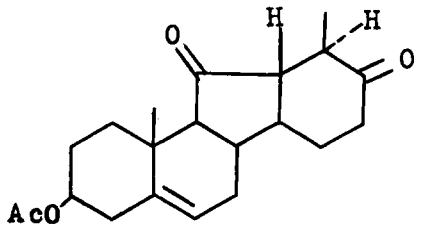
Using zinc powder in acetic anhydride saturated with HCl at 0°, α-acetoxy or α-haloketones have been reduced in high yields to completely saturated derivatives.[28] A new method of reduction of $\Delta^{1,4}$-3-ketones to Δ^{4}-3-ketones with lithium and biphenyl has been reported.[29]

Efficient methods for the stepwise degradation of a single carbon atom of the bile acid side chain have been developed. In one scheme, the essential step is the α-monobromination of cholanic aldehydes with iodine bromide in very high yield[30]; in another, it is the oxidation of α-hydroxy acids in high yield to aldehydes with sodium periodate under carefully controlled conditions.[31] It has been shown that the action of chromium trioxide on steroidal axial allylic alcohols under acidic conditions can lead not only to oxidation to a ketone, but also to epoxidation of the double bond on the same side of the hydroxyl group.[32] The reaction presumably proceeds through the formation of a chromate ester followed by transfer of oxygen to the double bond. The oxidation of a pregn-16-en-20-one with oxygen, trialkyl phosphite and a strong base has furnished a method for the preparation of 17α-hydroxy-pregn-15-en-20-ones.[33] The use of haloperoxidase of Caldariomyces fumago has led to new enzymatic methods of introducing halogen into the steroid nucleus.[34]

The structures of the adducts of ergosterol acetate and maleic anhydride, the first of which were isolated by Windaus and Lutringhaus in 1931, have been revised.[35] One is a normal adduct of endo configuration, in which the dienophile addition has occurred from the α-side. The others are produced by an addition at C-7 and concomitant abstraction of either the 9α or the 14α hydrogen to give the 7α-succinic anhydride derivatives of 3β-acetoxy-5,8,22-triene and 3β-acetoxyergosta-5,8 (14), 22-triene. Similar addition to the diene system with acrylonitrile and dimethyl diazodicarboxylate has been observed.[36]

Carbocyclic - The synthesis of 8α, 9β-estrone 3-methyl ether,[37] the elusive boat C-ring isomer, and 8α and 8α, 10α-19-nortestosterone[38] have been reported. Recent approaches to the transformation of steroids to their A-homo derivatives include the

solvolysis of some 19-hydroxyandrostanes to $\Delta^{1(10)}$-19-nor-A-homoandrostenes,[39] and the decarboxylation of a 1α, 2α-methylene-$\Delta^{4,6}$-3-ketopregnadiene to a $\Delta^{1(10),4\alpha(5),6}$-pregnatrien-4-one.[40] When A-homo-cholest-4α(5)-en-3-one was irradiated in t-butyl alcohol a mixture of 5α and 5β-vinyl-A-norcholestan-3-ones was obtained. B-nor-testosterone was obtained in an overall 30% yield by irradiation of 6,7 epoxytestosterone 17-acetate with light of 253.7 mμ[42]; a 7-ketone is obtained when the epoxide is irradiated with light above 310 mμ. A number of synthetic approaches to the C-nor-D-homo steroids have been reported. The intermediate 6 previously obtained from jervine, has been converted to C-nor-D-homoprogesterone and C-nor-D-homoestrone.[43] In an effort to prepare model compounds for the synthesis of cevine and veratramine, reactions of C-nor-D-homosapogenins have been examined.[44] The rearrangement of 14β-hydroxy-12β-tosyloxy steroids, epimeric 12-cholanol mesylates[45] and 12-oxotosylhydrazones[46] has led to additional C-nor-D-homo steroids. D-nor steroids have been obtained by base catalyzed rearrangements of 16β-mesyloxy-17α-hydroxy steroids bearing a 17β-acetyl group in which the ketone is protected.[47] Steroids possessing a 3,5 and 2,5 ethano bridge have been prepared by utilizing 5α-cyano steroids,[48] and 4,5-ethano steroids have been obtained by photochemical addition of ethylene to Δ^4-3-keto steroids.[49]



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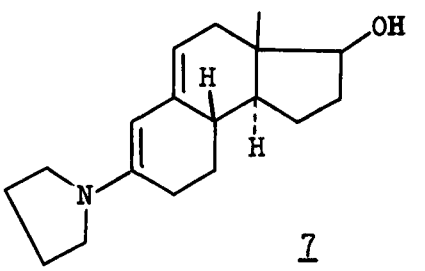

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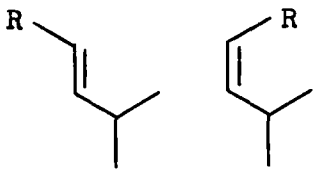
Heterocyclic - Recent developments in the total synthesis of heterocyclic steroids have been reviewed.[17] Among other heterosteroids which have been prepared and which resemble the natural hormones one should mention derivatives of 8-aza-19-norestraenes,[50,51] 5-azabisnortestosterone,[52] and 11-oxaprogesterone.[53] The reduction of 3-methoxy-6-methyl-6-azaestra-1,3,5(10),8(14)-tetraen-17-one with lithium, alcohol and methylamine leads to 6-azanortestosterone derivatives.[54] The reduction fails when ammonia is substituted for the amine. Several S-dioxide-3-thia steroids have been synthesized utilizing an intermediate prepared by condensation of 7 and methylvinylsulfone.[55] Some of the fused ring heterosteroids which have been prepared include the (2,3-c) thiophenes in the androstane series[56] and the (3,2-b) furanoestraenes.[57] The photolysis of steroidal nitrones has yielded oxaziranes.[58]

Substituted Steroids - The synthesis of 18-norestrone and the 18,19-dinor analogs of testosterone, progesterone, and desoxycorticosterone starting from 3-methoxy-17-

acetylgona-1,3,5(10),16-tetraene have been described.[59] A new route to 19-norsteroids involves removal of the methyl group in 3-hydroxy-1-methyl-estra-1,3,5(10)-trienes by oxidation to an aldehyde followed by abstraction of carbon monoxide with tris-(triphenylphosphine) rhodium chloride in refluxing benzene.[60] The synthesis of ± 18-methyl-19-norpreg-4-ene-3,11,20-triones[61] and 12-methylestratienes[62] resulting from previous total synthesis efforts have been reported. Birch reduction of 3-methoxyestra-1,3,5(10),15-tetraene-17-ol proceeds selectively on ring A, without reduction of the allylic system in ring D, to yield Δ^{15}-19-nor derivatives.[63] A method for the preparation of steroidal-4,6-dienes has been described which involves the dehydrobromination of a Δ^5-7-bromo steroid with an inorganic base in the presence of catalytic amounts of organic base and mercuric chloride.[64] It has been observed that, unlike the hydrogen bromide cleavage of a 1α-epoxy-3-keto steroid to a 2-bromo derivative, hydrogen fluoride causes rearrangement yielding a 4-fluoro-Δ^1-3-ketone.[65] Steroidal 17-allene acetates,[66] 17β-and 17α-mercaptoandrostanes,[67] 16-amino-17-hydroxy-20-ketopregnanes[68] and 3-nitroandrostane and 20-nitropregnanes[69] are substituted steroids which also have become available. The chemistry and synthesis of 19-oxygenated and 19-nor steroids have been reviewed.[70]

Plant Hormones and Steroids - Sterols. Contrary to previous conclusions, NMR data have indicated that the side chain orientation about the C-24 (28) bond is as in 8 in fucosterol and as in 9 for the avenasterols (isofucosterols).[71]



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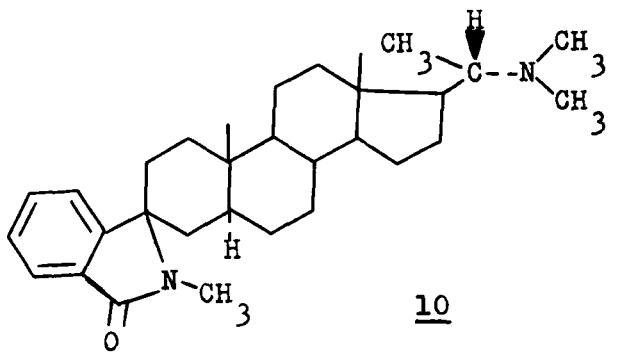

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Saponins and Bufadienolides. Michael addition of 1-acetoxy-5-nitro-2-methylpentane to cis-pregna-5,17(20)-dien-3β-ol-16-one furnishes adducts which readily lead to steroidal sapogenins. By using nitroacetates of the appropriate configuration stereoselective synthesis of kryptogenin, diosgenin, yamogenin and isoarthogenin have been effected.[72] Convallamarosid, a saponin with the sugar linked in the side chain, has been found.[73]

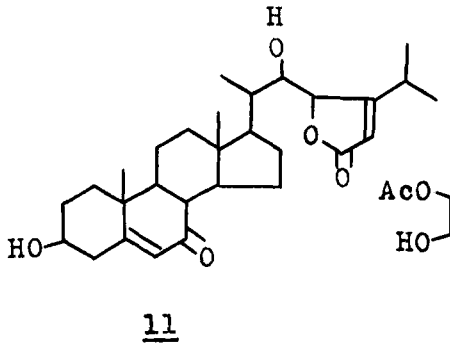
In plants, among the many polyhydroxypregnanes which have been isolated, the structures for purpurogenin,[74] digacetigenin,[75] stephanol,[76] drevogenin P,[77] cynanchogenin, sarcostin,[78] and holacurtin[79] and the constituents of the Chinese

crude drug "Wujiapi"[80] have been established. A synthesis of purpnigenin has been reported.[81] The new bufadienolides, bufagenin[82] and two naturally occurring tumor inhibitors, hellebrigenin 3-acetate and 3,5-diacetate from B. abbyssinica, [83] have been isolated and characterized.

Alkaloids. The configuration at C-17 in the formulation of jervine has been revised and a firm basis for the assignment of relative and absolute configurations to jervine, veratrobasine, 11-deoxojervine, veratramine and verarine has been established.[84] Spiropachysine, a major alkaloid of the leaves Pachysandra terminalis, has been shown to have the novel spirolactam structure 10[85]. The first example of ring E scission of the soladinine skeleton has been reported. Soladinine acetate reacts with cyanogen bromide to give an excellent yield of the 16-bromo-N-cyano derivative.[86] Using a general approach to steroidal sapogenins a synthesis of soladinine has been achieved.[87]

Fungi. The structure and absolute configuration of the steroidal fungal metabolite Wortmannin has been established[88] and the biogenesis of viridin from R-mevalonic acid has been affirmed.[89] By a combination of chemical and microbial transformation which introduces an 11-hydroxyl group into the helvolic acid skeleton, an interrelationship between fusidic and helvolic acid has been established.[90] The tentative structure 11 for atheridiol, the first steroidal sex hormone to be recognized in the plant kingdom, has been proposed.[91]

Invertebrate Hormones and Steroids - 12β-Hydroxypregna-4,6-diene-3,20-dione (cybisterol) and 21-hydroxypregna-4,6-diene-3,20-dione have been found to be protective materials secreted by the prothoracic protective gland of Mexican cybister species.[92] New invertebrate moulting hormones related to ecdysone, but varying in the position of stereochemistry of hydroxyl groups in the cholestane skeleton, have been isolated and characterized. Among them are amarasterone A and B, cyasterone, shidasterone,[93] makisterone A,B,C, and D,[94] ponasterones A (crustecdysone), B,C, and D,[95] deoxycrystecdysone,[96] and 5β-hydroxyecdysterone.[97] The partial synthesis of several of the insect hormones has been reported. Crustecdysone (20-hydroxyecdysone) has been obtained from progesterone,[98] whereas rubrosterone has been synthesized beginning with 17β-acetoxy-5β-androstane-3,6-dione[99] or with 12 derived from ecdysterone.[100] An ecdysone synthesis has been reported which utilized 13 in an oxidation with monoperpthalic acid to introduce the required 14α-hydroxy-7-en-6-one moiety.[101]



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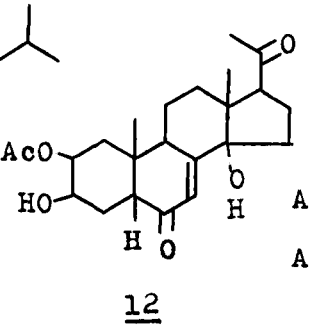

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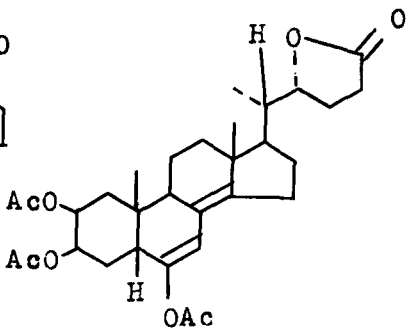

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Chapter 26. Reactions of Interest in Medicinal Chemistry

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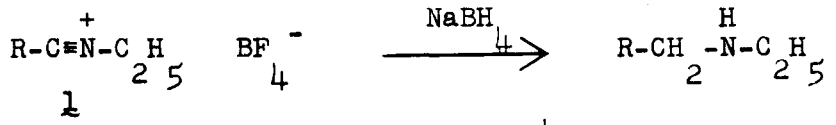
Carbon-carbon double bonds of α,β-unsaturated ketones are reduced by alkali metals in hexamethylphosphortriamide[1]. Yields and stereochemistry of the reduction are comparable to those obtained in liquid NH_3. The source of H for the reduction is proposed to be the N-methyl groups of the amide.

Activated Zn powder in Ac_2O saturated with HCl or HBr provides a convenient selective Clemmensen reduction of less-hindered keto groups in polyketonic systems[2].

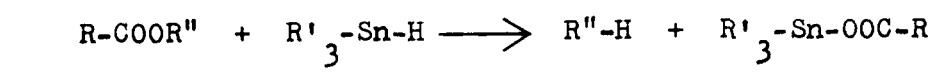
Treatment of nitriles with $(C_2H_5)_3O^+ BF_4^-$ results in formation of the cation 1 which is reducible to the secondary amine with $NaBF_4$ [3]:



Secondary and tertiary amides react with $(C_2H_5)_3O^+ BF_4^-$ to form imino ether fluoborates which are reducible with $NaBH_4$ to the amine in almost quantitative yields[4]. This method is useful when the molecule contains other reducible groups, such that $LiAlH_4$ or B_2H_6 are not satisfactory.

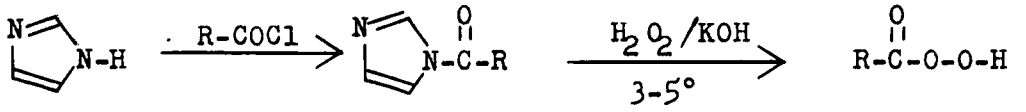
In the presence of catalytic amounts of Pd, acyl halides are decarbonylated smoothly to olefins, CO, and HX[5]. This new reaction is thus useful in degrading carboxylic acids by one carbon. A specially precipitated Ni in refluxing H_2O or aqueous dioxane reduces a wide variety of olefins, nitriles, carbonyl groups, and nitro groups[6]. Water acts as the H donor; alcohol solvents are not suitable. A prior preliminary report has been expanded to give details and scope of stereospecific hydrogenations with Ni modified by the addition of H_2O solutions of D- or L-tartaric acids[7]. Organo Li and Mg reagents add asymmetrically to carbonyl systems in the presence of (-)-sparteine[8]; sparteine is a bidentate ligand for R-Li to induce asymmetric ring opening of gem-dihalocyclopropanes to form optically active allenes[9].

A variety of esters is reducible by trialkyl tin hydrides[10]:



Use of R3-Sn-D or R3-Sn-T represents a new method of obtaining specifically deuterated or tritiated compounds.

A mild and general method of preparation of peroxy acids is illustrated[11]:



R may be alkyl, alkenyl, aromatic, or substituted aromatic.

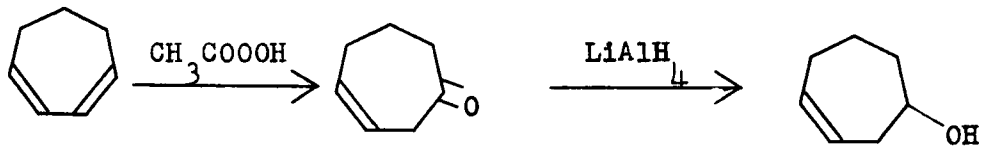
The difficulty in manipulation of Ag_2CO_3 (an excellent and specific oxidant of primary and secondary alcohols to aldehydes and ketones) is obviated by precipitation of the Ag_2CO_3 on Celite[12].

Co(II) salts are catalysts for cleavage of 1,2-glycols with O_2 in aprotic, polar solvents[13]; the yields of aldehydes are good. The method is adaptable to preparation of acids without isolation of the intermediate aldehydes.

Aldehydes react with CN^- and activated MnO_2 in acidic alcohol to form esters or free carboxylic acids[14]. Non-conjugated aldehydes are converted to the free acid rather than to the ester. In the presence of H_2O, trichloroisocyanuric acid reacts with certain ethers of the type $R\text{-}CH_2\text{-}O\text{-}R'$ to form esters $R\text{-}COO\text{-}R'$[15].

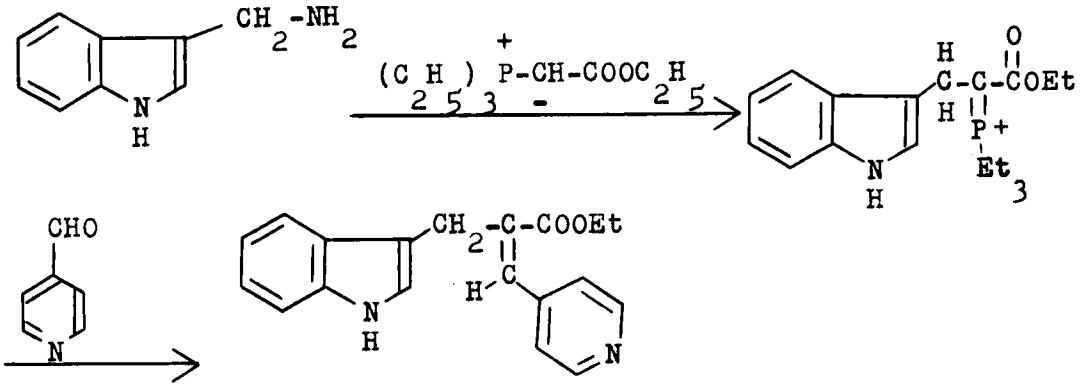
Treatment of tertiary methylamines with Pt black at ambient temperature in O_2 atmosphere yields an N-formyl secondary amide which may be hydrolyzed to the secondary amine[16]. N-Ethyl- and N-benzylamines are not attacked. Tertiary amines and commercially available organic hydroperoxides give excellent yields of amine oxides in the presence of vanadium salts and other group VB and VIB metal catalysts[17].

Dienes react with peracetic acid to form a monoepoxide which, upon reduction with $LiAlH_4$, is converted to a homoallylic alcohol[18]:



Alkyl and aryl disulfides, when treated with N_2O_5, give high yields of sulfonic anhydrides[19]. Diethyl azodicarboxylate forms a 1:1 adduct with mercaptans which, when treated with a second mercaptan, gives a good yield of the unsymmetrical disulfide[20].

Mannich bases may be converted to alkylated phosphorane systems which undergo the Wittig reaction to form β-arylacrylic acids having an α-functional group[21]:



Benzyl esters are preparable by passing O_2 through a solution of a methylbenzene, a carboxylic acid, $Pd(OAc)_2$, $Sn(OAc)_2$, and an alkali metal carboxylate[22].

Primary and secondary acids, on treatment with $Pb(OAc)_4$ and a $Cu(OAc)_2$ catalyst, give high yields of olefins[23]:

$$R\text{-}CH_2\text{-}CH_2\text{-}COOH \xrightarrow[Cu(OAc)_2]{Pb(OAc)_4} R\text{-}CH{=}CH_2 + CO_2 + 2H^+ + 2e^-$$

These decarboxylations can be carried out thermally at 80° or photochemically at 30° with the same result. Amides react with carboxylic acids or alcohols in the presence of $Pb(OAc)_4$ to form carbamates and ureas[24]:

$$R\text{-}CONH_2 + \begin{matrix} R'COOH \\ \text{or} \\ R''\text{-}OH \end{matrix} \xrightarrow{Pb(OAc)_4} \begin{matrix} H \\ R\text{-}N\text{-}COOR' \\ \text{or} \\ R\text{-}N\text{-}CO\text{-}N\text{-}R'' \\ H \quad\quad H \end{matrix}$$

γ-Oxo acids are oxidatively decarboxylated by PbO_2 to α,β-unsaturated ketones. The reaction appears to be general, but best yields are realized from α- or β-substituted acids[25].

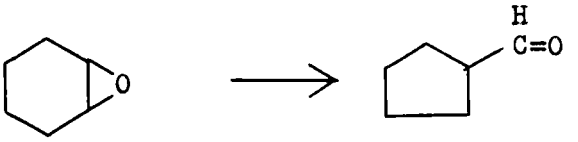
β-Dicarbonyl systems are C-alkylated in the presence of Tl^+ with no side reactions. The Tl^+ reagent permits either O- or C-acylation with acyl halides[26]. Phenol salts of Tl^+ react with acyl halides to give excellent yields of phenolic esters[27]; Tl^+ salts of carboxylic acids react with acyl halides to form the asymmetric anhydride. Aryl and secondary alkyl Grignard reagents can be coupled with TlBr[28].

Mg amalgam converts γ- or δ-haloketones to cyclanols[29]; γ-haloketones form cyclobutanols which are otherwise difficultly attainable.

α-Ethoxy organo Cd or Zn reagents react with a variety of carbonyl systems to form ethyl ethers possessing β-functionality[30].

Allylic coupling of acetylenes is accomplished by use of lithio-1-trimethylsilyl propyne[31]; there is minimal tendency for isomerization to allene systems.

Epoxides react with LiBr and tri-n-butylphosphine oxide to form carbonyl systems[32]:

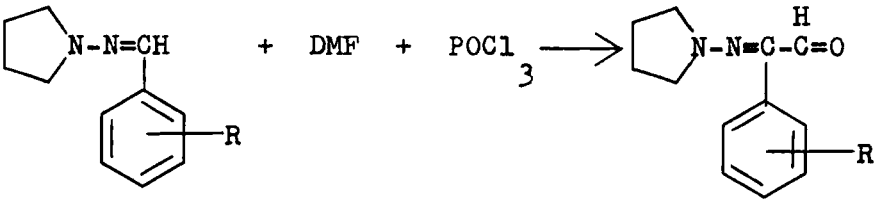


When $LiClO_4$ is used, ketones are formed with no ring contraction.

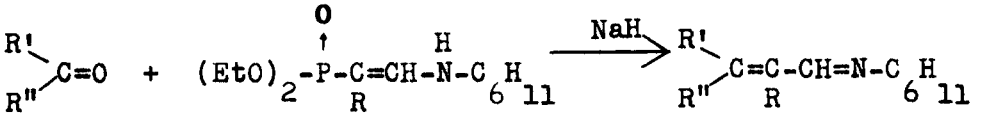
9-Borabicyclo[3.3.1]nonane participates in many hydroboration reactions as does diborane[33]; it is simple to prepare and is stable in air and toward heat. Organoboranes react at 0° with ethyl bromoacetate in the presence of K t-butoxide[34]; two-carbon homologation of the starting olefin is achieved. Rates of reaction of organoboranes with CO at atmospheric pressure and room temperature or lower is greatly enhanced by lithium trimethoxyaluminohydride[35]. Organoboranes undergo rapid reaction with I_2 in the presence of NaOH, to form primary iodides. This suggests that other organoborane reactions may be catalyzed by NaOH[36].

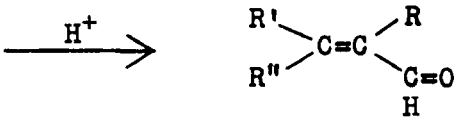
Under the influence of Pd, Rh, or Ru salts, aryl, methyl, and carboxyalkyl groups add to olefins[37]. The reaction is catalytic in the metal salt if $CuCl_2$ is used as a reoxidant; it is insensitive to O_2 and H_2O.

Aldehyde hydrazones may be viewed as aza-enamines; accordingly, benzaldehyde hydrazones undergo a Vilsmeier formylation[38]:



Formylolefination of aldehydes and ketones results in α,β-trans-unsaturated aldehydes having two more carbons[39]:



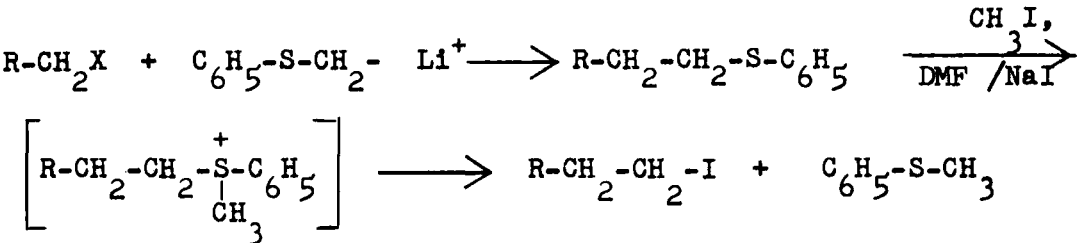


Dioxolanes derived from α-haloaldehydes, when treated with KF in ethylene glycol, undergo dehydrohalogenation without halogen interchange. This is a useful route to a great variety of α,β-unsaturated aldehydes[40].

α-Lithio sulfinamide derivatives react with aldehydes and ketones to form β-hydroxysulfinamides, which on thermolysis form olefins via a cis-elimination[41]. In parallel reactions, esters are converted to ketones. Methyl- and α-methylene ketones react with ethyl formate under base catalysis to form the α-formyl system which, upon treatment with p-tosyl azide, yields an α-diazoketone[42].

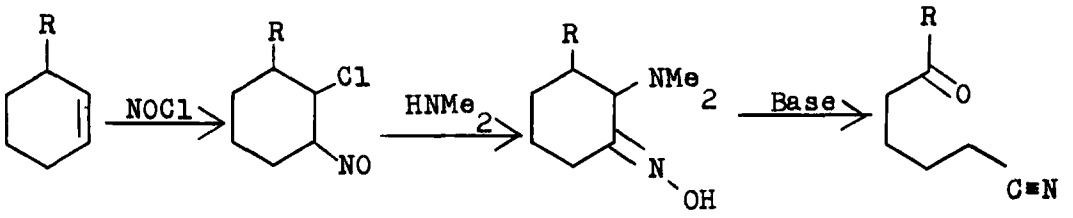
3-Ketosteroids are selectively converted to their dimethylketals by tris-(triphenylphosphine) rhodium (I) chloride and CH_3OH[43]. Ketonic groups elsewhere in the steroid system are unchanged.

Primary halides may be homologated by a two-step procedure[44]:



Replacement of the Li reagent with an analogous Cu one is preferable for homologation of allylic halides. Treatment of primary or secondary alcohols with tri-n-octylphosphine in CCl_4 or CBr_4 gives high yields of the primary or secondary halide with inversion of configuration[45].

Oximes, α-oximinoketones, and α-hydroxyoximes react with phenylacetic acid in the presence of dicyclohexylcarbodiimide to form the oximino ester[46]. At pH 5 and room temperature, H_2O-soluble carbodiimides convert hydroxamic acids to amines[47]. Cyanoaldehydes and -ketones are attainable by conversion of olefins to α-dimethylamino oximes and subjecting these to a Beckmann fragmentation[48]:

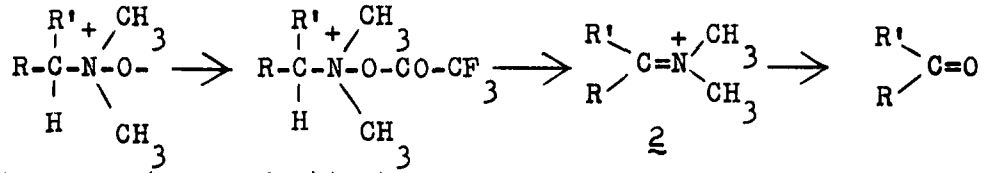


The reaction has been performed on rings from C_5 to C_{12}.

N,N'-unsymmetrically disubstituted piperazines are attainable by catalytic hydrogenation of the appropriate enamine of N-alkylpiperazine[49]. Enamines oxidized with $Tl(OAc)_3$ form α-acetoxyketones in yields generally superior to those obtained by oxidation of the free ketone[50]. Allylamines on treatment with KNH_2 on alumina isomerize to form high yields of the enamine[51].

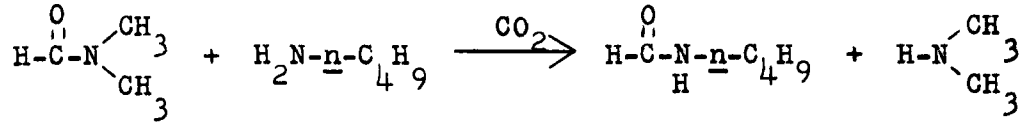
Phenylacetylene has been converted to an ynamine by reaction with dimethylamine and $Cu(OAc)_2$ in a stream of O_2[52]. Ynamines react with a variety of reagents to form pyrrocolines, 2- and 4-quinolones, unsaturated amidines, cyclic sulfones, and 4-membered cyclic enol ethers[53].

Mild treatment of N-oxides with trifluoroacetic anhydride yields immonium trifluoroacetates; the immonium structure may be hydrolyzed to a carbonyl group[54]:

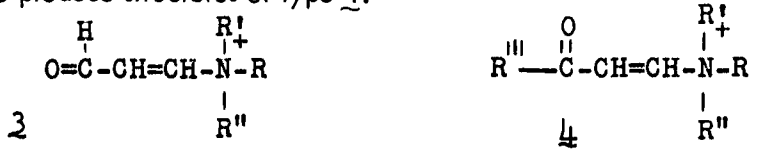


Structure 2 (R = R' = CH_3) has been considered the intermediate in Mannich reactions with $H_2C\tilde{O}$ and $NHMe_2$; it underwent a facile reaction with a variety of carbonyl systems, and it provided better yields than the classical Mannich procedure.

Certain amides undergo transacylation with amines in the presence of CO_2[55]:



Propiolaldehyde reacts with salts of tertiary amines to form the hitherto unknown N-(β-formylvinyl) ammonium salt 3[56]. Ethynyl ketones react analogously to produce structures of type 4.



3 4

Amides are readily converted into imidate fluoborate salts by triethoxonium fluoborate. The imidate salts are readily converted into amidines in better overall yields than by older methods[57].

Fluoride ion from tetraethylammonium fluoride acts as a strong base in aprotic solvents; it mediates α-elimination of HX from CX_3H to generate a carbene, and it catalyzes β-elimination of HBr from β-phenethyl bromide to afford styrene in almost quantitative yield[58].

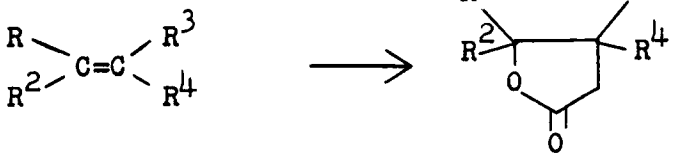
Treatment of a nitrile with an equivalent amount of 100% formic acid in a bomb permits hydrolysis to the pure amide in almost quantitative yield[59].

Chloroacetyl groups can be removed from amino functions by use of thiourea in ethanol[60]. The products are the amine HCl which is usually soluble in the reaction medium and pseudothiohydantoin which precipitates.

Reaction of olefins with Br_2 or I_2 and a Lewis acid in the presence of a nitrile which also acts as a solvent gives rise to 3,3-disubstituted -3,4-dihydroisoquinolines[61]. These systems are not preparable by the Bischler-Napieralsky method.

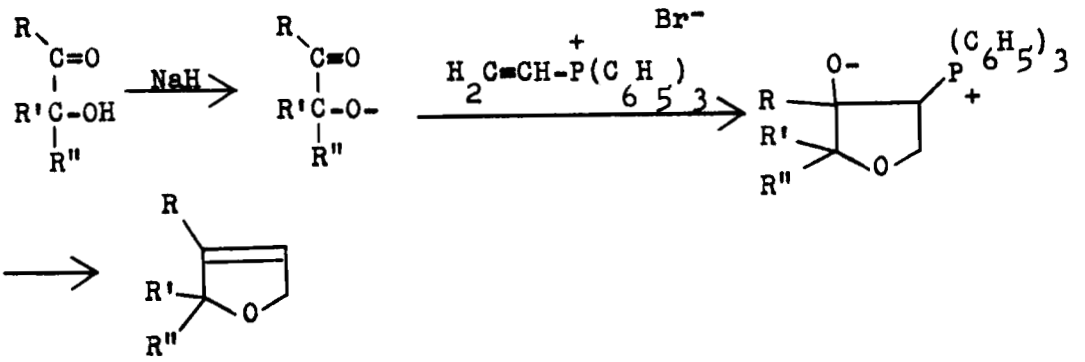
Diazoacetic acid azide has been prepared[62]; it is highly explosive, but it can be distilled. It is highly useful in preparation of a variety of pyrazole derivatives.

γ-Lactones are formed from olefins and Ac_2O or KOAc in the presence of $Mn(OAc)_3 \cdot 2H_2O$[63]:

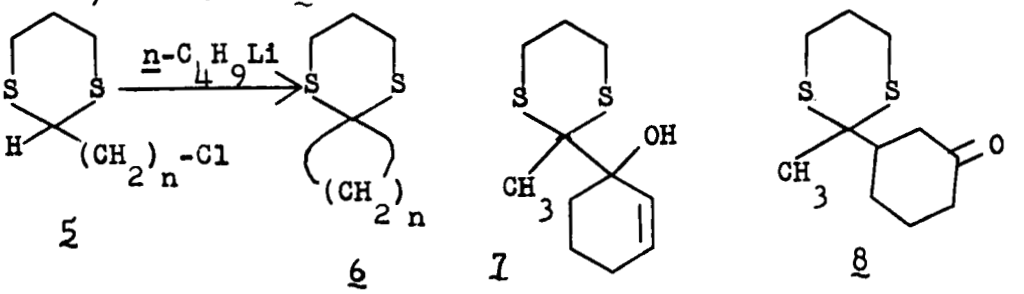


Tetrahydropyran-2-yl isocyanate, preparable from 2-chlorotetrahydropyran and AgCNO, reacts with amines to form monoalkyl ureas or unsymmetrical dialkyl ureas, and with alcohols to form alkyl carbamates[64].

2- And/or 3-substituted 2,5-dihydrofurans are preparable from α-hydroxyketones and vinyltriphenylphosphonium bromide[65]:



2-(ω-Chloroalkyl)-1,3-dithianes 5 are convertible to cycloalkane trimethylene thioketals 6[66]:



Hydrolysis of 6 leads to a ketone; this method was applied to preparation of cyclic 1,3- and 1,4-diketones. 2-Lithio derivatives of 2-methyl-1,3-dithianes are synthetically equivalent to C-nucleophilic carbonyl groups; depending upon the reaction conditions, they will add to 2-cyclohexenone in a 1,2- or a 1,4- manner[67] to form 7 or 8. Cleavage of the dithiane ring can lead to a variety of side chain ketonic cycloalkane or cycloalkene systems.

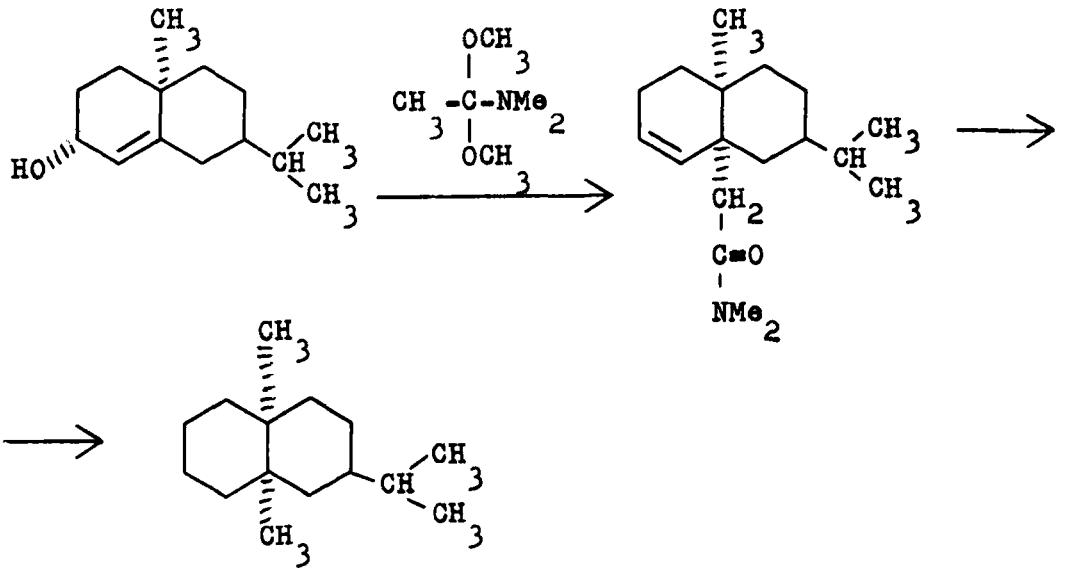
Treatment of primary alcohols with iodosobenzene produces excellent yields of aldehydes[68]; 1,4-butanediol is converted to tetrahydrofuran by iodosobenzene. Reaction of 1,2-cis-dicarboxylic acids with this reagent leads to the anhydride.

A simple procedure leading to methyl 2,5-dienoates utilizes allyl halides, acetylene, CO, and CH_3OH. The reaction occurs at room temperature and atmospheric pressure and is based upon in situ formation of a catalytic complex of Ni with thiourea. The products contain a cis-double bond conjugated with the ester group.[69]

A three-step synthesis for substituted adamantanes utilizes 4-ketocyclohexane-1,1-dicarboxylic esters[70]; the adamantane product, bearing keto and carbalkoxy groups, lends itself to further molecular modification.

Stereoselective introduction of an angular methyl group utilizes the Claisen rearrangement and the recently reported decarbonylation of aldehydes

with tris-triphenylphosphine rhodium (I) chloride[71]:



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Chapter 27

Pharmaceutics, Pharmacokinetics and Biopharmaceutics

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Introduction - The availability of the drug from the dosage form depends on physical chemical factors, chemical interactions and a priori predictions and confirmations or release in biological systems.

Drug Dissolution, Diffusion, and Interactions - These phenomena continue to be of paramount importance in the overall picture of drug absorption by the body. Dissolution rates of benzoic acid in surfactant solutions were much less than those anticipated on the basis of solubilization data. However, the ratios of these dissolution rates in the surfactant solution to those in pure solvent were found to be much greater under static conditions than those under stirred conditions, suggesting a difference in the dissolution mechanism[1]. Dissolution rates of benzocaine were determined in water and polysorbate using both the propeller-driven stirrer and the rotating disc method providing a unique method for distinguishing dissolution mechanisms[2]. Furthermore, the data in the above study confirms the fact that the rotating disc model rather than the diffusion layer model is operative under stirred conditions and that the Danckwert's model is operative under static conditions[3]. Dissolution from constant surface pellets into micellar solutions follows first order kinetics[4] and clarifies an earlier communication which proposes the existence of a considerable time lag before the occurrence of steady state dissolution in micellar solutions[5]. The in vitro dissolution rates of phenacetin and phenobarbital in human gastric juice and in hydrochloric acid containing polysorbate 80 were compared. The increase in dissolution rate observed as a result of the presence of the surfactant was attributed to surface tension lowering and not to solubilization[6]. A new apparatus for dissolution studies of tablets and capsules is described which can be automated by connecting the pump to a flow cell in a suitable spectrophotometer[7]. Variations in ionic strength and hydrostatic head had no significant effect on the rates of diffusion of 4' aminopropiophenone through a silastic membrane[8]. The transport of aminophenones through silastic membranes is consistent with the partitioning from the diffusing solution into the membrane, diffusion within the membrane, and subsequent partitioning in the desorbing solution[9]. The apparent diffusion constants through a silastic membrane for a series of barbiturates and phenylalkylamines were correlated with chloroform-acetate buffer partition coefficients. Fick's law for solid polymeric membranes is operative with respect to the concentrations of uncharged organic compounds[10].

The importance of the crystal form of the drug is illustrated when anhydrous-ampicillin is shown to be significantly more water soluble than the trihydrate below the transition point and its solubility is inversely related to temperature[11]. Agitation accelerates the diffusion controlled

processes of dissolution and crystallization of the stable and unstable crystal forms of cortisone acetate in aqueous suspensions leading to the formation of a high proportion of large particles[12]. Subsequent results reveal that crystal growth is mainly initiated by polymorphic transformation. Lattice energies, heats of wetting and solution of the different crystal forms are thought to be the rate of growth controlling factors[13]. Eight distinct polymorphs of phenobarbital were isolated and had distinguishable infrared absorption spectra and X-ray powder diffraction patterns[14].

Flow properties of powders and particle size considerations have a direct bearing on the availability of the drug. Angle of repose values calculated for various mixtures of starch and salicylic acid indicate a direct dependence on the microscopic structure of the agglomerates[15]. A strong negative correlation exists between the flow rate of lactose granules and the proportion of particles less than 100 mesh size[16]. A refined recording powder flow meter for the quantitative evaluation of non-uniform flowing formulations was developed which prints out the time in hundredths of a minute for pre-selected weight increments of powder[17]. A relatively simple apparatus and procedure for measuring the static friction coefficient of powders in simple beds is also described[18].

A panel of experts recently recommended that the pharmacological effects of any new orally administered drug having a low solubility in water be investigated in relation to particle size[19]. Particle size reduction of reserpine in a solid state dispersion led to increased dissolution and absorption rate and possibly increased availability of reserpine[20]. Flow rates of sodium borate and boric acid from acrylic and brass funnels were calculated, indicating that large particles discharge steadily from the funnel but flow behavior varies in the region below critical size. The rate depends largely on outlet diameter, outlet length, and cone angle of the funnel[21]. The effect of particle size of boric acid, aspirin, and magnesium alumino-silicate on the fluidity and packing properties of starch mixtures produced remarkably small values for the angle of repose and porosity of the starch-magnesium aluminosilicate mixture, suggesting that the latter may be withdrawing moisture from the starch particle[22]. A method has been developed for the determination of the particle size distribution of griseofulvin and phenothiazine suspensions by measuring the increase in dielectric constant as the particles settle out of a narrow zone in the suspension. The results agree well with those obtained by the standard Andreasen's sedimentation method[23]. In order to quantitate the variance in active ingredient due to improper powder mixing, two theoretical equations were developed for spherical and for cubic particles[24]. Various ratios of salicylic acid to starch were mixed in two different types of blenders, the coefficient of variation calculated and compared to a theoretical value obtained from Stange's equation.[25]

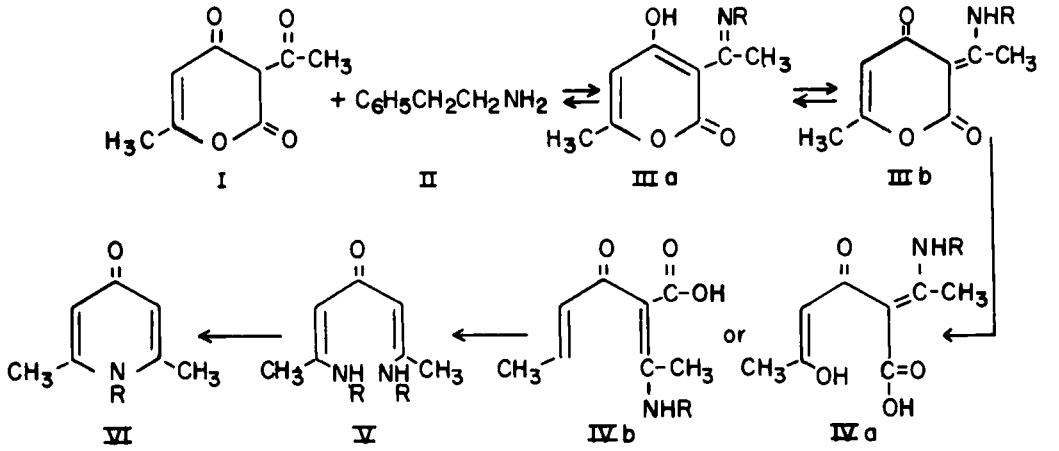
The importance of dosage form is illustrated where tablets containing triamterene and hydrochlorothiazide caused approximately twice as much excretion of hydrochlorothiazide and three times as much tri-

amterene as capsules containing the same proportions of the drugs[26]. An improved method of coacervation has been worked out which gives high percentage in vitro release of sulfamerazine from gelatin micro-capsules of different wall thicknesses[27]. A correlation was established between drug release from chloramphenicol capsules as reflected by deaggregation and dissolution rates, and by plasma levels. The availability of the drug varies considerably with the commercial lot used[28].

Humidity has an adverse effect and heating a favorable one on the tabletting properties of microcrystalline cellulose[29]. Pressures ranging from 100 to 4,000 kg/cm^2 were applied to powders and the relaxation modulus changed sharply with the increase in pressure particularly in the lower pressure range[30]. Dissolution rates of phenobarbital tablets from different manufacturers were calculated using a modified oscillating tube method and the results indicated considerable discrepancies among the various lots[31]. The effect of binder concentration, tablet hardness, and storage conditions on the dissolution rate of phenobarbital from tablets was established[32]. A definite proportionality exists between the in vivo and in vitro disintegration times of enteric coated tablets. However, between preparations containing different substances the proportionality factor may differ significantly[33]. A linear relationship exists between a calculated thickness of the coating of enteric coated tablets and the in vitro disintegration time measured by different methods, regardless of the size and content of the tablets[34]. Significant factors affecting drug availability from coated tablets were shown to be the drug-to-diluent ratio and the composition of the diluent[35]. The polyvinylpyrrolidone and salicylic acid dissolution rates from compressed mixtures were consistent with the theoretical model describing the dissolution rate behavior of polyphase mixtures. The polyvinylpyrrolidone data were in agreement with the simplest of these models describing dissolution of non-barrier solid from a mixture of two non-interacting solid phases[36]. Important factors influencing the in vitro release of steroids from topical preparations into the receptor phase were shown to be the solubility in the vehicle and the partition coefficient of the steroid between the vehicle and the receptor phase[37]. The comparison of the rates of drug released from the planar surface of a compressed polymer matrix with the theoretically predicted values showed that as long as the integrity of the hydrated polymer was maintained the release of the drug was diffusion controlled[38]. The applicability of a polymer-drug interacted system in the design of dosage forms was investigated and the release rates of the polymer-drug salt were comparable in drug availability to those of the free drug or its hydrochloride[39]. Dissolution rate studies show that the surfactant lysolecithin significantly increases the solubility of griseofulvin, glutethimide, and a new diuretic compound in aqueous media. However, reduction in particle size does not necessarily increase dissolution rate possibly due to the establishment of a charge, upon milling, which causes aggregates to be formed in the media[40]. Dissolution rates of griseofulvin were calculated in four non-ionic surfactants and in a polyoxyethylene glycol showing in every case but the latter a significant increase in dissolution rate as compared to that for the drug in water[41]. The dialysis of a number of drugs was studied to show that in many cases the anionic

form of a drug is bound to the membrane. Two salicylate ions may be bound per glucose unit in the cellulose membrane. Ephedrine hydrochloride was dialyzed considerably faster than the corresponding base due to the fast dialysis of the chloride ion[42].

Drug Stability - The theoretical bases for formulation of stable drugs depend upon knowledge of the thermal solvolysis of the drug in solution as a function of pH, concentration and excipients. Goto et al, after a prior study of the Schiff base formation of 3 (1-phenethylamino) ethylidene-6-methyl-3H-pyran-2,4-dione, III, from dehydroacetic acid, I, on reaction with β phenethylamine, II[43], have determined that III is stoichiometrically transformed to the reactive 2,6-Bis-(phenethylamino)-2,4-heptadiene-4-one, V, in the presence of excess II with no significant concentration of the intermediate IV experimentally or kinetically observable[44]:



The log k-pH rate profile of the decomposition of 2-phenylindandione-1,3 was spectrophotometrically investigated in buffered aqueous solutions and the heats of activation determined[45]. The profile demonstrates a minimum at pH 9 and implies that the higher pH-independent solvolysis below a pH of 6 may be attributed to water attack on the undissociated species or the kinetically equivalent hydrogen ion attack on the anionic species. Increasing rates of solvolysis with increasing pH values above 9 may be attributed to hydroxyl ion attack upon the enolate anion. Homochlorcyclizine, HCC, dihydrochloride in aqueous solution degrades to p-chlorobenzhydrol, p-chlorobenzhydryl chloride, p-chlorabenzophenone and N-methylhomopiperazine[46]. The doubly protonated HCC^{+2} is attacked by water or the monoprotonated species is attacked by hydrogen ion to cleave the bond between the p-chlorobenzhydryl group and the nitrogen atom. The result is a pH independent solvolysis below a pH of 2 and negligible solvolysis above a pH of 5. Aqueous solutions of cyclamic acid, $C_6H_{11}NHSO_3H$ slowly degrade to sulfuric acid and cyclohexylamine at rates proportional to hydrogen ion concentration. These rates are more highly accelerated in solvents of increasing alcohol content[47]. N-methyl-4-phenyl-4-carbethoxypiperidine hydrochloride (meperidine hydrochloride) shows specific hydrogen and hydroxyl ion catalyzed

solvolysis with a minimum at pH 4[48]. Buffer catalysis by dihydrogen phosphate ion and a positive primary salt effect was observed. The hydrolysis rates of acetaminophen prodrugs, $CH_3CONH-C_6H_5-OCOOR$, at pH 7.4 in phosphate buffer alone or with 1% human plasma descreased as the electrophilic character of R decreased and increased with plasma concentrations[49]. The trichloroethyl carbonate ester shows specific hydroxyl ion catalyzed and water catalyzed solvolysis. The solvolysis is also catalyzed by plasma and intestinal fluid[50]. The hydrolysis of carbonate esters of salicylic acid were only slightly accelerated by 2% human plasma whereas butyl and hexylcarbonate ester solvolyses were more significantly accelerated by pseudocholinesterase and α-chymotrypsin systems[51]. This suggests that carbonates with 4 or 6 carbon alkyl chains better fit the active sites of these esterolytic enzymes. Glycylglycine ethyl ester decomposes to glycylglycine and 2,5-piperazinedione in the pH range 8.7 to 10.8[52]. The rates of ester solvolysis may be attributed to the specific hydroxyl ion catalyzed hydrolysis of the protonated and neutral ester. Ring closure is dependent on a hydroxyl ion catalyzed solvolysis and a solvent attack on the neutral ester.

Glutethimide (2-ethyl-2-phenyl glutarimide) exhibits specific base catalysis in the pH range 1.5-8.0[53]. The hydrolysis of 3-carbomethoxy-1-methylpyridinium cation in the pH region 8.0-9.8 is specific hydroxyl ion and buffer catalyzed and the rate is significantly decreased by 8-chloro-theophyllinate anion due to the formation of a spectrally observable complex[54]. The suppression of the photodecomposition of menadione with various electron donors is due to the formation of resistant charge transfer complexes[55]. Dimethylsilicone solutions of menadione are more thermally stable than with corn oil, although solutions in the latter are more resistant to photodegradation[56]. Sucrose monolaurate solutions show first order solvolysis kinetics to free lauric acid below the critical micelle concentration[57]. First order kinetics are not observed above the CMC and the presence of laurate anion forms mixed micelles and inhibits hydroxyl ion attack. Chlorothiazide undergoes parallel routes of equilibration in aqueous solution to N-(2-amino-4-chloro-5-sulfamoylphenylsulfonyl) formamide in aqueous alkali and 5-chloro-2, 4-disulfamoylformanilide in acidic media. Both routes lead to the final product, 4-amino-6-chloro-m-benzenedisulfonamide[58] and log k-pH profiles are available[59]. The specific hydroxy ions catalyzed hydrolysis of the anionic 5-halouracils produces barbituric acid and uracil[60]. Hydroxyl ion attack on the undissociated halouracil produces non-chromophoric products that may result from rapidly degrading isobarbituric acid. The log k-pH profiles are presented and buffer catalysis by phosphate and buffer was observed. The 5-halouridines undergo similar solvolysis[61] and produce unstable ribosylbarbituric acid in alkali. The antiviral 5-trifluoromethyl-2'-deoxyuridine, F_3TdR, hydrolyzed by hydrogen ions to 5-trifluoromethyluracil, F_3T, and deoxyribose[62]. The F_3T is readily hydrolyzed to 5 carboxyuracil in alkali which may be decarboxylated at elevated temperatures. The F_3TdR is readily hydrolyzed to 5-carboxy-2'-deoxyuridine by hydroxyl ion attack even in the neutral region.

Lachman[63] has reviewed the chemistry and use of antioxidants and chelating agents as stabilizers in liquid dosage forms. Nonisothermal stability techniques for the determination of rate constants as a function of temperature on the basis of a single kinetic run have been applied to the hydrolysis of N-acetyl-p-aminophenol, procainamide[64] and dry horse serum cholinesterase[65]. Heterogeneous kinetics are needed in the exact study of drug stability in the solid state. The oxidative degradation of ascorbic acid has been analyzed to consider the rates of diffusion and reaction of the reactant gas with the drug as the solid[66,67].

Pharmacokinetics and Biopharmaceutics - The quantification of the distributions of acute doses of a drug and its metabolites in the multicompartmental complex organism and its rates of metabolism and excretion as functions of dose are necessary prerequisites for the determination of its biological availability from dosage forms. The achievement of a constant urinary excretion rate of a metabolite may be considered indicative of saturation of an enzymic system. There must be an appreciable negative intercept when the linear portion is extrapolated back to zero time for this system and for the similar model of constant rate intravenous infusion. The mathematical significance of the lag time for the one-and two-compartment body models has been given[68]. Krüger-Thiemer[69] has discussed non-linear factors that affect dose-concentration relations due to drug dissolution, solubility and saturable active absorption processes; due to multicompartmentalization and saturable protein or tissue-binding sites; due to metabolic processes where enzymic sites are saturable; and due to tubular secretory processes that may be saturable. Other clinical factors such as age, wakefulness, body position, diet enzyme induction, concomitant drugs can also perturb the pharmacokinetics[70]. Accumulation kinetics as a function of dose, half-life and dosage interval again have been mathematically described[71,72,73]. An elegant intellectual design of the optimum dose ratio among triple sulfas has been based on relative solubilities, biological efficacies, and pharmacokinetic[71] factors of absorption and elimination[73]. A plot of the log rate of excretion of total drug against time ultimately exhibits a linear section with a slope equal to the sum of the rate constants which govern the first order processes of drug elimination when a zero order process is occurring simultaneously [74]. Pharmacokinetics in applications of the artificial kidney must consider the events of protein binding, lipid solubility and metabolism that occur in the body during extracorporeal treatment of blood as well as relative flow rates[75]. Flow rates and perfusate volumes affect the rate constant for drug elimination from perfused organs[76]. The excellent book by Röpke and Riemann[77] offers a compendium of analog computer programs and techniques applicable to pharmacokinetics. The pharmacokinetics of amphetamine-like compounds and their release from dosage forms in vivo under controlled acidic[78] and fluctuating[79] urine conditions have been examined using an analog computer. Urinary pH and volume output also influences the excretion of ephedrines in man[80] and their absorption and metabolism have been pharmacokinetically evaluated[81].

Anisotropine methylbromide shows a sustained excretion rate in man, possibly indicative of a "deep" compartment and is orally absorbed[82].

Pharmacokinetic constants for the absorption and elimination of pralidoxime have been determined in man[83]. A pharmacokinetic model for flow, lipid solubility, protein binding and saturation-limited metabolism of thiopental has permitted the a priori prediction of bodily distribution consisten with experiment[84]. Imipramine and its metabolites are rapidly distributed in the rat and renally and biliary excreted with enterohepatic circulation[85]. Mathematical models have been established for the pharmacokinetics of neurohypophysial and related peptides[86]. The oral administration of 2,3,5, triiodebenzoic acid in goats and a cow by whole-body radioactivity retention showed a rapid distributive and subsequent exponential elimination phase with the metabolites formed by deiodination[87]. Bishydroxycoumarin shows dose-dependent first order elimination in man but not in other species[88] and has been assigned to dose effects on elimination[89].

Probenecid inhibits the renal excretion of indomethacin[90] and it has been suggested that high serum levels of penicillin and others are not only due to diminished tubular secretion but to decreases in distributive volumes[91]. The metabolism of isoproterenol is largely due to 3-O-methylisoproterenol parenterally and the sulfuric acid ester orally[92] in the dog. The pharmacokinetic analysis of the tritiated drug implied that metabolism was fast and that the prolonged tachycardia induced by oral medication results from slow absorption. The formation of 4-hydroxyacetanilide from acetanilide in rabbits shows enzyme saturation kinetics[93]. The half-lives and distributions of terephthalic acid[94] and mercury compounds[95] have been studied in the rat. It has been claimed that performance scores are highly linearly correlated with the "tissue" compartment of the two compartment body model for LSD[96]. Sulfonamide pharmacokinetics have been investigated in the rat and are similar to man[97]. However, acetylsulfonamides have higher excretion rates than their corresponding sulfonamides and suggest a different excretory mechanism. Many drugs may competitively interfere with the protein binding of sulfonamides and affect the pharmacokinetics[98]. Acetylsalicylic acid in man has salicylic acid as its exclusive metabolite[99]. Its pharmacokinetics conforms to the two compartment body model and is not dose dependent. The evidence for dose dependent pharmacokinetics of salicylic acid has been summarized recently[49]. The fact that concomitant administration of salicylamide and salicylate decreases glucuronide formation in man is consistent[100]. Pheniramine is N-demethylated in man and chlor- and brompheniramine in that order have increased metabolism[101]. The main metabolite of sulfaphenazole in humans is the N-glucuronide[102]. Those of sulfamethomidine are the N^4 acetyl and the N^1 glucuronide[103]. 4-Butylaminoantipyrine is dealkylated in four animal species to 4-aminoantipyrine and its acylated metabolite with a minor amount of 4-(2-butenylamino) antipyrine formed, probably through dehydration of the usual penultimate hydroxylation route of butyl aryl ethers[104]. Bucetin is metabolized to glucuronides in rabbits[105]. The pharmacokinetics of griseofulvin in man[106] and its metabolism in the dog[107] have been evaluated. An excellent book on analytical methods for drug metabolites has been written by Hirtz[108].

Ballard[109] has reviewed the factors affecting the absorption rates

of drugs administered subcutaneously and intramuscularly whereas Lemanowicz has outlined the factors involved in the gastrointestinal absorption of drugs[110]. The influence of pharmaceutical excipients and adjuncts on drug absorption has been summarized by Munzel[111]. It has been shown that methods based on the one compartment-body model give unacceptable estimates of absorption rates; a non-equilibrated tissue compartment must be evaluated to be rigorous[112]. If a significant degree of metabolism takes place in the gut or in the liver, comparison of area under blood curves as criterion of comparison for drug availability among oral and other routes is invalid[113]. The area under the blood curve was shown to be linearly related to the product of the dose, the half-life and the reciprocal of the body weight in man for novobiocin[114]. The Thiry-Vella fistula dog provided a quantitative and reproducible system for the evaluation of drug absorption from the intestinal mucosa[115].

Overturn time in the goldfish is a pharmacologic end point for biologic membrane permeation and has been used in kinetic studies with pentobarbital and ethanols[116]. The absorption of 3-Methoxy-N-methylmorphinan, pKa 8.0, from the rat stomach at pH 2.0, has been assigned to the formation of ion pairs of significant lipid solubility[117]. The absorption of naldixic acid from the rat stomach was consistent with the pH-partition hypothesis[118]. Lincomycin is more readily absorbed in rats as the 7-esters than the 2-esters due to their greater rates of intestinal hydrolysis[119]. The effect of crystal size and morphology on chloramphenicol palmitate absorption are confirmed[120]. Aspects of the effects of dissolution rate on drug availability have been characterized by mathematical expressions[121]. The laxative oxyphenisatin produces significant inhibition of glucose absorption in the rat and man[122]. Prodrug carbonate esters of salicylic acid are as readily absorbed as aspirin[123]. Gastric emptying of phenol red in rats is a first order process and is delayed by sodium deoxycholate[124].

Perfusion of hydroxylated benzoic acids, benzaldehydes, methylbenzoates through the small intestine of anesthetized rats showed increased absorption rates with the number of hydroxyl groups with ortho isomers being absorbed fastest[125]. The values of the logarithm of the ratio of the absorption rate constant for a substituted phenyl derivative to the unsubstituted is a constant and independent of the series. Absorption rates of drugs from the rat small intestine are modified by complex formation between sodium p-aminosalicylate and sulfisoxazole, sulfisomidine, sulfamethoxypyridazine and in the complexing pairs sodium salicylate-caffeine and hydroxyethyltheophylline-nicotinamide, aminopropylon-sulfamethizole, aminopropylon-sulfisomidine and riboflavin phosphate with sulpyrin, caffeine and 4-aminoantipyrine[126] other similar studies were conducted[127,128]. Complexes affect the rate of transfer of model drug compounds through artificial lipoid barriers due to relative lipid solubilities of complexes and free drugs[129]. Caffeine complexes *in vivo* do not necessarily follow the observed absorption pattern observed with an in vitro lipid barrier[130].

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Chapter 28. Physicochemical Parameters in Drug Design

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Introduction - From the literature one can see that the application of computer techniques to the development of quantitative structure-activity relationships is becoming increasingly prevalent and successful. Although this review does not include those papers which were cited by Hansch last year,[1] more research was reported using the linear-free-energy related[2] (semi-empirical) models than using the mathematical[3] (empirical, de novo) models. The predictive power of these models and their utility in delineating factors important in biological response continue to hold great promise in drug design. It should be emphasized, however, that these techniques were not developed as nor claimed to be a panacea in selecting molecules for synthesis and evaluation. Rather, they constitute useful tools which, when used with common sense and discretion, can provide a rational guide for synthetic work. As the reliability of the activity data improves and the models are refined, one can expect more meaningful results using these methods. The reader's attention is called to an extensive discussion of the application of regression analyses and molecular orbital calculations in medicinal chemistry[4] and a review of these methods by Cavallito.[5]

Quantitative Structure-Activity Relationships
Mathematical (Empirical) Model - Employing the method of Free and Wilson, Beasley and Purcell have reported the successful prediction of the butyrylcholinesterase inhibitory potency of 1-decyl-3-(N-ethyl-N-methylcarbamoyl)piperidine hydrobromide.[6] Three years after the predicted biochemical response was published,[7] this compound was synthesized and evaluated biochemically. The observed response was found to be quantitatively near the predicted value.[6]

Recently Ban and Fujita have applied this mathematical model to the norepinephrine uptake inhibition of several sympathomimetic amines.[8] It was shown that the response parameters could be analyzed by constant and additive activity contributions of the substituents and the parent phenylethylamine structure. These results suggest that the conformation of the parent structure at the receptor site is not markedly changed by the addition of the substituent groups studied. The use of the logarithms of the biological response parameters was suggested in this type of analysis since this eliminates meaningless negative activities.[8]

Linear-Free-Energy Related (Semi-Empirical) Model - More efforts have been directed toward attempts to correlate the biological activities of congeners with their measured physicochemical properties and to delineate the importance of each. Hansch and his colleagues continue to lead the development in the use of substituent constants in structure-activity studies.[9]

Partition Coefficients - Hansch and co-workers have continued to obtain good correlations between substituent partition parameters and biological response.[10] In the series of various organic compounds studied, the major variation in structure-activity relationships was associated with the relative lipophilic character of the substituents as defined by their octanol/water partition coefficients.[10] Examples of these correlations are given in eq 1 and eq 2, where MR

$$\log MR = -0.184(\log P)^2 + 0.641 \log P + 1.478 \qquad n = 13 \quad r = 0.974 \quad (1)$$

$$\log MR = 0.452 \log P + 1.767 \qquad n = 7 \quad r = 0.954 \quad (2)$$

represents the relative metabolic rate of the compounds and log P is the difference in the logarithms of the partition coefficients of the unsubstituted parent compound and its substituted analog. The value of n represents the number of compounds used in the regression and r is the correlation coefficient of the regression. The ideal lipophilic character (P_o) for amines undergoing metabolism by crude monamine oxidase was defined by log $P_o = 2$. This value also holds for the formation of glucuronides by alcohols and benzoic acids and the formation of hippuric acid by benzoic acids.[10]

Lien and Hansch have shown a strong correlation between ratios of drug metabolism by microsomal subfractions and partition coefficients.[11] This illustrates a "non-specific" correlation since several different enzymes are undoubtedly involved in the metabolism of the 10 unrelated drugs studied. Equation 3 gives the correlation. In eq 3, $R_{(s/r)}$ is the

$$\log R_{(s/r)} = -0.101 \log P + 0.859 \quad n = 10 \quad r = 0.885 \quad (3)$$

ratio of enzyme activity in the smooth and rough particles of endoplasmic reticulum.[11]

Application of regression analysis, using partition coefficients, to the binding of organic compounds by synthetic polymers has been made by Hansch and Helmer.[12] Based on prior work,[13] their studies of the nylon and rayon binding of derivatives of aniline and acetanilide from an aqueous solution have shown that the amount of compound bound is related

to the octanol/water partition coefficients of the compounds. This dependence of binding on partitioning properties to synthetic macromolecules parallels that found for a variety of biopolymers.[12]

Further correlations (r = 0.96) between the binding of a variety of 42 small organic compounds to bovine serum albumin and their partition coefficients were obtained by Helmer, Kiehs, and Hansch.[14] The linear relationship in this series again illustrates the non-specific nature of the process. It was also observed that the change in optical rotation of the albumin caused by the adsorbate is proportional to the logarithm of the partition coefficient.[14] Based on the work of Gordon and Jencks,[15] Hansch and co-workers have used partition coefficients to indicate the dependence of the denaturation of T4 phage DNA upon the lipophilic character of the denaturant.[14] These conformational changes per unit of hydrophobic character seem to parallel the hydrophobic binding of organic compounds by macromolecules.[14]

In other studies, Lukens and Horsfall have shown a linear relationship between the inhibition of glycolate oxidase by a series of phenoxyacetic acids and the hydrophobic binding properties of the compounds.[16] This study would indicate that these compounds bind, in part, hydrophobically to the enzyme.[16] Büchi and co-workers have observed definite relationships between the partition coefficients of 4-aminobenzoic acid esters and local anesthetic activities.[17,18] An increase in the partition coefficients of these compounds (decrease in water solubility) was accompanied by an increase in the local anesthetic response. Dittert, et al., have suggested that the lipid and water solubilities of acetaminophen prodrugs studied, rather than their enzymatic rates of hydrolysis, probably control the availability of acetaminophen following oral administration.[19] Certainly lipophilic character is a major influencing factor in eliciting the response from these drugs.

Electronic and Steric Constants - In addition to hydrophobic parameters, homolytic and steric constants were successfully used in the structure-activity studies of 1,3-benzodioxole synergists of carbaryl in flies.[20] Although the hydrophobic character of the synergists is quite important (log P_o = 4), electronic substituent effects paralleled those for homolytic arylation and improved the correlation.[20] Use of Hammett's electronic constant (σ) and Taft's steric parameter (E_s) gave a better correlation when used with the hydrophobic substituent constant. Observations in this study have suggested a possible mechanism of action of the synergists on the molecular level.[20]

McFarland has suggested the use of group dipole moments (μ) and electronic polarizability (α) parameters in addition to hydrophobic and Hammett substituent constants (eq 4).[21] In

$$\log 1/C = k_1\pi^2 + k_2\pi + k_3\sigma + k_4\mu^2 + k_5\alpha + k_6 \quad (4)$$

eq 4 C represents the molar concentration of drug necessary to elicit a defined biological response, π is equal to log P, and k_1, k_2, k_3, k_4, k_5, and k_6 are constants generated by the regression. Addition of these parameters has yielded better correlations when applied to the inhibitory rate constants of E. coli by chloramphenicol and nine of its analogs. Other variations of this equation (e.g., deletion of σ or π^2) give improved correlations in several systems.[21]

Clayton and Purcell have reported excellent correlations using dipole moments, amide group moments, and Taft's polar substituent constants (σ^*) along with hydrophobic parameters when applied to an homologous series of molecules.[22] A regression of the butyrylcholinesterase inhibitory potencies of these mono(carbamoylpiperidino)decanes gave eq 5 in which

$$pI_{50} = -0.119\pi^2 + 1.205\pi + 0.687\sigma^* + 3.533$$
$$n = 6 \quad r^2 = 0.998 \quad (5)$$

pI_{50} is the negative logarithm of the molar concentration of compound necessary to effect 50% inhibition. The predictive utility of this method was demonstrated: the calculated pI_{50} value of 1-decyl-3-(N-ethyl-N-methylcarbamoyl)piperidine hydrobromide was within the experimental error of the observed value. A comparison of the results of this semi-empirical method with those[7] of the empirical mathematical method indicated a better fit using the Hansch method.[22]

Hansch and co-workers have also used σ, σ^*, E_s, and pK_a values to give excellent correlations with relative metabolic rate parameters.[10] Hansch and Helmer have also used pK_a values to give improved correlations between the partition coefficients (log P) of 17 aniline derivatives of varying basicity and their binding to rayon and nylon.[12] Addition of ΔpK_a (ΔpK_a = pK_a Aniline - pK_a Substituted Aniline), which is analogous to Hammett's σ constant, gave an increase in correlation coefficient from 0.719 to 0.974 and 0.789 to 0.970 for nylon and rayon binding, respectively.[12] In addition, Fujita has observed that the changes in auxinic activities of 1,2,3,4-tetrahydro-1-naphthoic acid and its homologs, atropic acid derivatives, and 3,4-dihydro-1-naphthoic acid and its homologs may be expressed as a function of the changes in lyophobic/lyophilic character, steric structure, and electronic structure of the auxins.[23]

Quantum Mechanical Indices - Cammarata has suggested the use of molecular orbital methods in accounting for certain drug-receptor interactions to correlate drug structure with activity.[24] Using the atomic orbital coefficient, c_N, associated with the amino nitrogen and the total electronic charge on this nitrogen, q_N, as substituent parameters, he has obtained excellent correlations with the activities of benzoyl (eq 6)

$$\log (1/C_r) = 2.04c_N + 146.69q_N - 1549.99q_N^2 + 17.63 \qquad r = 0.97 \qquad (6)$$

and phenyl (eq 7) derivatives of sulfanilamides against E. coli. In eq 6 and eq 7, C_r is the minimum inhibitory concentration of the compound against E. coli.[24] He has also suggested that the Hansch π parameter may be considered a measure of drug receptor interactions that fit the category of frontier-controlled reactions.[24] Other molecular-orbital derived parameters (such as the energy of the highest occupied molecular orbital, HOMO, π-electrophilic superdelocalizability, and energy level differences) have also been suggested for consideration in correlating drug-receptor interactions.[24]

$$\log (1/C_r) = 2.26c_N + 122.36q_N - 456.63q_N^2 - 3.19 \qquad r = 0.99 \qquad (7)$$

Neely, White, and Rudzik have applied the Hückel molecular orbital technique to estimate the different electronic parameters in attempting to deduce which index correlates with the analgesic activity of a series of imidazolines.[25] The most significant index for correlation appeared to be the HOMO, a relative measure of the ability of an electron to be transferred to an acceptor molecule. Using the linear-free energy model of Hansch, the analgesic activities (log ED_{50}) were regressed against the π values and HOMO parameters. Although the π values account for 53% of the activity (eq 8),

$$\log ED_{50} = 0.655\pi^2 - 1.00\pi + 0.455 \qquad r^2 = 0.531 \qquad (8)$$

addition of the HOMO parameter leads to explanation of 94% of the variance (eq 9).[25] There was, however, no correlation

$$\log ED_{50} = 0.945\pi^2 - 1.850\pi + 7.90(\text{HOMO}) - 5.117 \qquad r^2 = 0.944 \qquad (9)$$

between HOMO and biological activity when regressed alone. This may indicate again that the lipophilic factor is the critical influence in controlling the concentration of drug

at the receptor site where the intrinsic reactivity takes over to elicit the response.[25]

Molecular orbital methods have also been used by Cammarata and Stein in the study of cholinesterase inhibitors.[26] The π net charge, nucleophilic π superdelocalizability, and electrophilic π superdelocalizability parameters were applied to the acetylcholinesterase inhibitory potencies of some 3-hydroxyphenylammonium derivatives with considerable success. Correlations indicate the inhibitory response depends upon the strength of the hydrogen bond of the 3-hydroxy group of the inhibitor and esteratic site on the acetylcholinesterase.[26]

The relationship between the structure and activity of amphetamine and its substituted derivatives has been investigated by quantum chemical methods.[27] Nagy and Nador have found that the central exciting effect increases with a decrease of the negative charge on the second carbon of the benzene ring. This activity was present only in the derivatives whose dipole moment stemming from the conjugated part was considerably less than the dipole moment of the conjugated part in noradrenaline. These two parameters have permitted theoretical predictions of the most active amphetamine derivatives.[27]

Reactivity indices for each of the ring carbons of phenylsulfide acetic acid and eleven chlorinated derivatives have been calculated by the linear combination of atomic orbitals method.[28] Cocordano has shown that the auxinic activities of these compounds correlated well with the sum of the reactivity indices of the 3- and 6- carbon atoms.[28] In addition, Mainster and Memory have proposed that alternative electronic indices, founded upon the Hückel molecular orbital theory, may be used to characterize chemical carcinogens.[29] Based upon the use of superdelocalizability to estimate relative chemical reactivity, these indices have been used to characterize molecular carcinogenicity accurately.[29]

The use of the sum of the highest occupied molecular orbital and the lowest empty molecular orbital as a measure of the molecular "electronegativity" has been applied to quinolinemethanol antimalarial studies by Purcell and Sundaram.[30]

Kier[31-34] and Neely[35] have applied molecular orbital theory to calculate the preferred conformations of various compounds and to propose the nature of their respective receptor sites. Kier's calculation of the preferred conformation of cortisol[31] has recently been confirmed experimentally by Cole and Williams using infrared and nuclear magnetic

resonance techniques.[36] Other studies by Kier have included calculations on histamine and a theory of its dual activity,[32] serotonin,[33] and ephedrine isomers.[34] Neely has applied this technique to study the preferred conformation of α-D-glucopyranose.[35] Kier and Whitehouse have used interatomic distances of some anti-inflammatory agents and inflammagenic amines to give possible insight into their common receptors.[37]

Proton Magnetic Resonance - Cammarata and Allen have begun to explore the possibility of correlating the proton magnetic resonance spectral properties of sulfanilamides with their observed bacteriostatic activities.[38] In the congeneric series studied, however, free rotation about the SO_2-N bond has frustrated these attempts and indicated the need for model systems in which rotation is prohibited.[38]

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Chapter 29. Alkaloids

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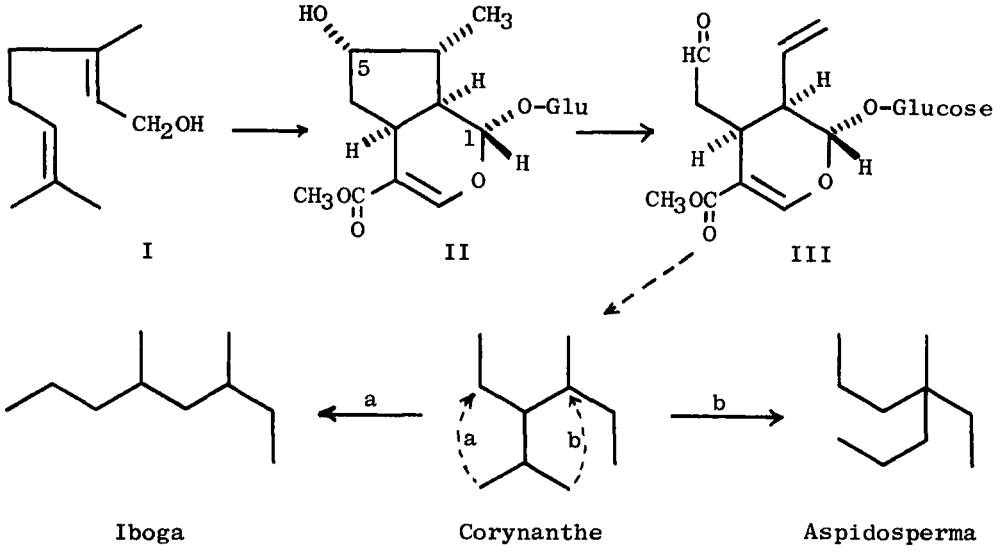
Reviewing a field as active as the chemistry and biochemistry of alkaloids in the space allotted must necessarily result in the omission of considerable material of merit. It was the author's intention to cover the advances of most interest to the general reader with the recognition that it would involve an unavoidable personal bias.

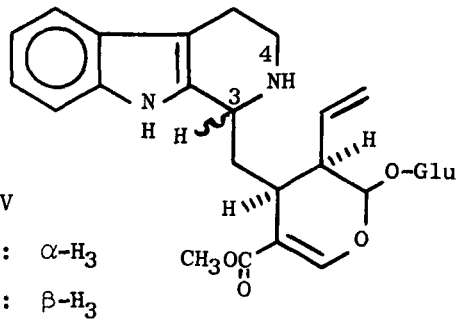
Volume 10 in the series "The Alkaloids" edited by R.H.F. Manske[1] appeared of which almost half was devoted to the steroid alkaloids of the Solanum and Veratrum groups. The remainder includes sections on Erythrophleum, Lycopodium, benzylisoquinoline, indole and Taxus alkaloids.

The single major advance of the year was in the greater understanding of the biosynthesis of the indole alkaloids. The monoterpenoid origin for the non-tryptamine portion, already demonstrated with the incorporation of radiolabelled mevalonate and geraniol (I) pyrophosphate was reviewed[2] the previous year. During the past year the postulated cyclopentane intermediate which would yield the terpene skeleton of the three types of indole alkaloids in Vinca rosea was shown to be loganin (II)[3,4] whose stereochemistry was established in three laboratories[5-7] and its biosynthesis confirmed in Vinca rosea.[4] The synthesis of radioactive (doubly-labelled) secologanin (III) and its feeding to the same plant resulted in the incorporation of tracers without scrambling.[8] Evidence for



an early nitrogen containing intermediate in the biosynthesis was obtained with the discovery of strictosidine (IV) (stereochemistry not established) in Rhazya species[9] and its presence subsequently demonstrated in V. rosea by dilution analysis starting with radioactive tryptophan and loganin (II)[10].

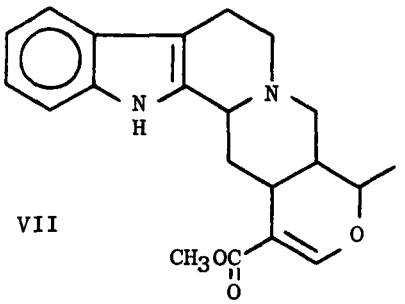
A mixture of the radioactive isomers, vincoside (V) and isovincoside (VI) (enantiomeric at C-3 with one isomer very likely strictosidine) prepared from secologanin of known stereochemistry and tryptamine, when fed to Vinca resulted in the isolation of radioactive ajmalicine (VII), vindoline (VIII), catharanthine (IX) and perivine (X)[11]. Dilution analysis experiments with [5-^{3}H]-loganin (II), radio-inactive secologanin (III) and the vincoside-isovincoside mixture resulted in the trapping of activity into these compounds in good yields. Subsequently, N^4-acetylvincoside was isolated from the glycoside fraction in a good yield of 19 mg/1.5 kg of fresh V. rosea[12] and vincoside (V) was shown to be converted to the three types of indole alkaloids. Isovincoside (VI) was not an effective precursor.



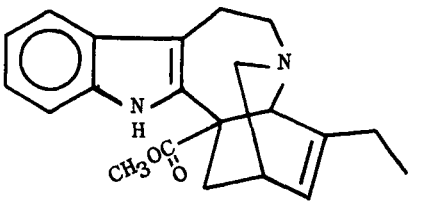
IV

V: α-H_3

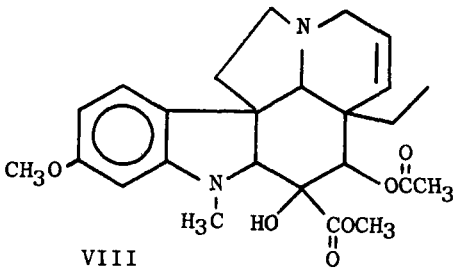
VI: β-H_3



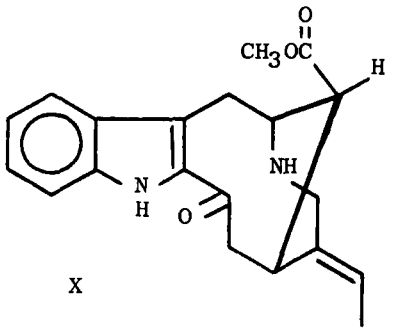
VII



IX

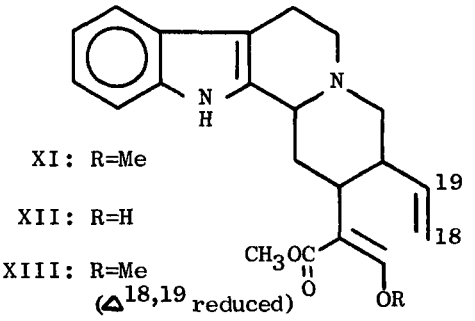


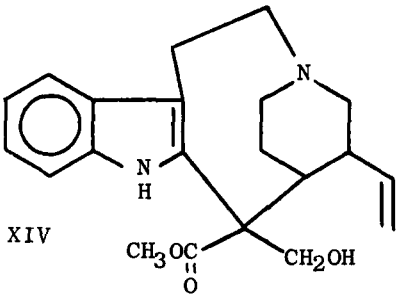
VIII

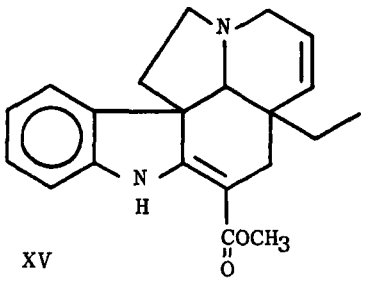


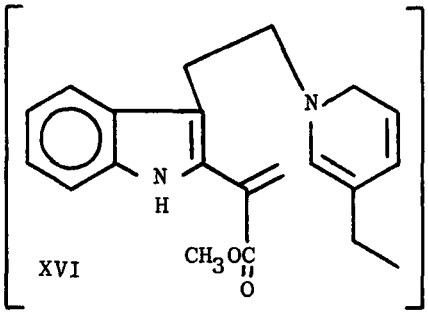
X

The interrelationship between these major types of alkaloids was shown, elegantly, in a time study of their appearance in 1-300 hr seedlings of V. rosea.[13] The Corynanthe types XI, XII and XIII appeared in the second day followed by ajmalicine (VII) and stemmadenine (XIV), two additional Corynanthe-type alkaloids. After three days, tabersonine (XV), an Aspidosperma type, appeared and was followed in seven days by vindoline (VIII) and the Iboga alkaloid, catharanthine (IX). This alkaloid distribution in the seedlings is quite different from that in 3-month old plants. Readministration of the seedling alkaloids and measuring their incorporation gave support for the biosynthetic scheme in Fig. 1. In another study interconversion in 6-month old plants was also reported[14] and the result is consistent with this pathway.









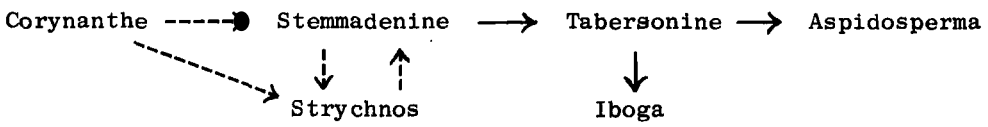
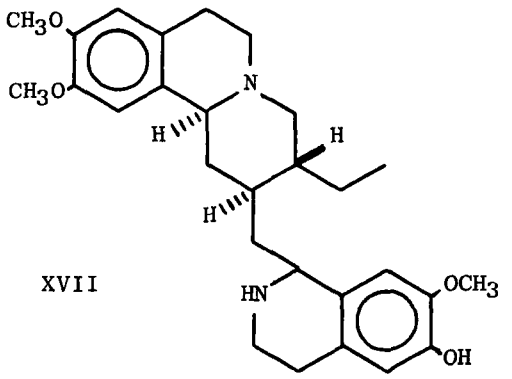


Figure 1

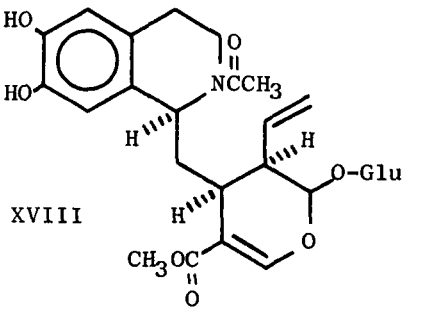
Qureshi and Scott[15] in addition showed the same *in vitro* interconversion by refluxing stemmadenine (XIV) in acetic acid, thus establishing the oxidation level of the precursor, and postulating a common acrylic ester intermediate (XVI) which necessarily restricts additional changes to more elaborately substituted alkaloids to subsequent steps. A noteworthy finding in support of this restriction was the high (4.8%) biochemical conversion of tabersonine to vindoline.[13] Certain other limitations on the mechanism for the biosynthetic relationship have been obtained from tritium loss or retention in going from specifically tritiated geraniol and mevalonate[16] to the final bases. An alkaloid from *Tabernamontana cumminsii*[17] representing a new group of indole alkaloids has the basic skeleton of the postulated intermediate XVI, while the secamines[18] from *Rhazya* species are examples of the dimeric form.

Two reports on the stereochemistry of the tetracyclic[19] and the pentacyclic[20] oxindole alkaloids appeared which with the earlier communication[21] provides physical correlations for assigning the total spatial structure.

The biosynthesis of the Ipecac alkaloids as illustrated for cephaeline (XVII) and ipecoside (XVIII) involves geraniol (I) and loganin (II) for the non-tyramine derived portion, apparently in a manner similar to the indole alkaloids.[22]

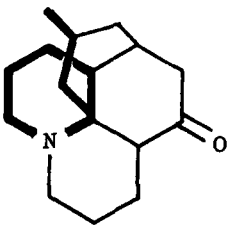


XVII



XVIII

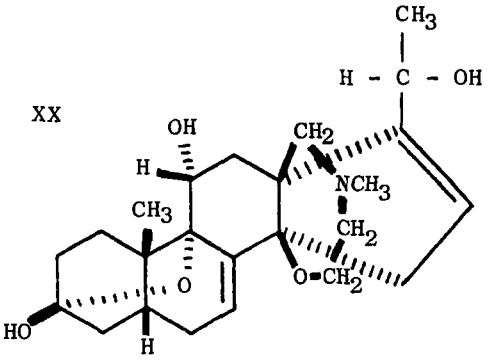
Lycopodine (XIX) from *Lycopodium flabelliforme* was found to incorporate label from lycine-2-^{14}C and -6-^{14}C and to fit an origin from two isopelletierine units ($C_5H_9NHCH_2COCH_3$)[23], a scheme at variance with a polyacetate origin. Two chemical syntheses of this alkaloid have appeared[24,25] as communications.

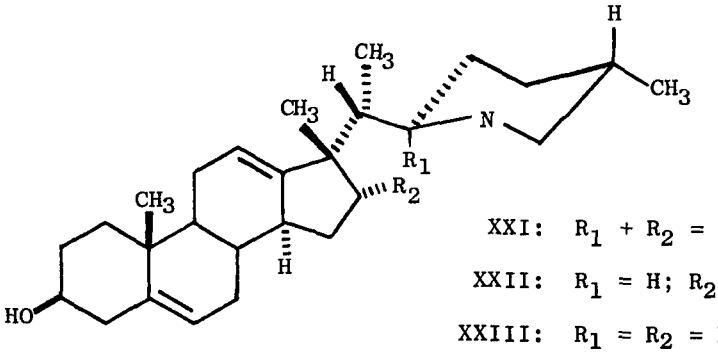


XIX

The X-ray determined structure of batrachotoxinin A (XX), the more stable water addition product of pseudobatrachotoxin and one of a number of cardiotoxic substances from the Colombian arrow poison frog, Phyllobates aurotaenia, has been reported.[26] It represents a novel steroid structure containing an N-methylethanolamine unit. Another new type of steroid alkaloid represented by veramine (XXI)[27], veralkamine (XXII)[28], and veralinin (XIII)[29] was obtained from Veratrum album subsp. lobelianum. The structures were established by physical and chemical methods including X-ray analysis of veralkamine hydroiodide.[30] This new class has the structure and stereochemistry that would arise by a biochemical Wagner-Meerwein process in which the C-18 methyl group of a normal steroid would migrate to C-17 by a β-side 1,2-rearrangement.

XX



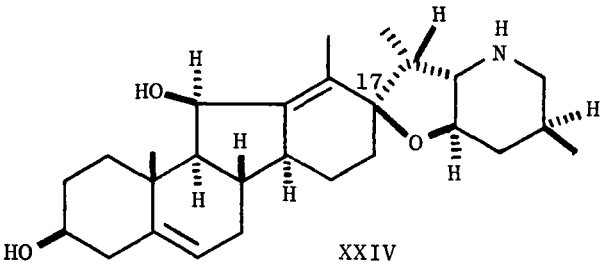


XXI: $R_1 + R_2 = O$

XXII: $R_1 = H$; $R_2 = OH$

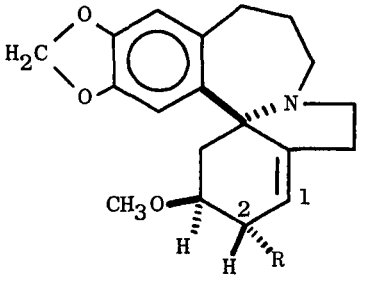
XXIII: $R_1 = R_2 = H$

The stereochemical structure of the Veratrum alkaloid veratrobasine (XXIV) was obtained by X-ray studies[31] and this result required that the C-17 configuration in jervine be revised after it was demonstrated that veratrobasine and jervine-11β-ol were one and the same. This also established the absolute configuration for jervine, 11-deoxojervine, veratramine and verarine.[32]



XXIV

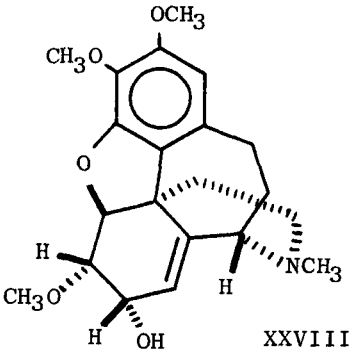
To the increasing group of substances derived from a phenethylisoquinoline precursor, a previously unreported type related to the Erythrina alkaloids has now been added. These "homoerythrina" alkaloids, schelhammerine (XXV), schelhammericine (XXVI) and schelhammeridine (XXVII)[33] were isolated from Schelhammera pedunculata; a plant belonging to the same tribe (Uvularieae) of the family Liliaceae as do the other plants yielding the "homo-" group of alkaloids.



XXV: R = OH

XXVI: R = H

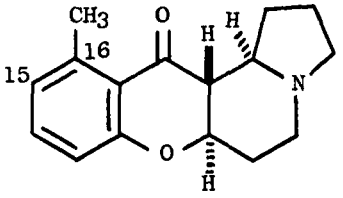
XXVII: $\Delta^{1,2}$ instead



XXVIII

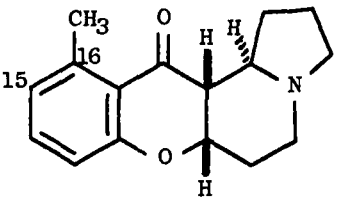
The structure for (+)-Kreysiginine (XXVIII), a homomorphinan from Kreysigia multiflora Reichb. was established by physical and chemical methods[34,35] and by X-ray diffraction.[36] The alkaloid CC-21 from Colchicum cornigerum is enantiomeric with (+)-kreysiginine.[35]

The first structural information about alkaloids from the family Elaeocarpaceae unfolded a new type of base possessing a indolizidine ring. Five examples are known at present: (±)-elaeocarpine (XXIX) (X-ray analyzed), (±)-isoelaeocarpine (XXX), (+)-elaeocarpiline (XXXI), (-)-isoelaeocarpiline (XXXII), and (+)-isoelaeocarpicine (XXXIII).[37,38] Structure assignment was by physical methods (n.m.r., i.r., u.v. and mass spectrometry), as well as by chemical interconversion. Elaeocarpus polydactylus Schl. yielded XXIX, XXX and XXXIII and E. dolichostylis Schl. gave XXXI and XXXII.



XXIX

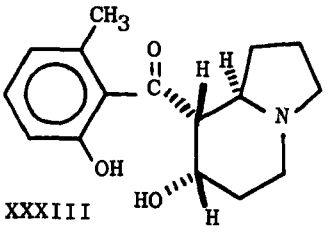
XXXI (15,16 bond reduced)



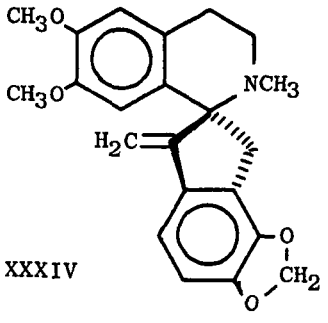
XXX

XXXII (15,16 bond reduced)

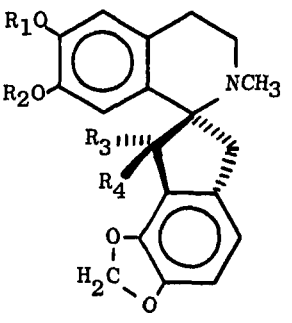
A total synthesis of (±)-ochotensimine (XXXIV), one of the first discovered "spirobenzylisoquinoline" alkaloids has been accomplished.[39] The total number of these alkaloids was increased to five with the addition of fumaricine (XXXV), fumariline (XXXVI) and an unnamed one (XXXVII).[40] Structure assignments were made by physical methods and in particular by the use of the nuclear Overhauser effect (N.O.E.).[41]



XXXIII



XXXIV



XXXV: $R_1 = R_2 = CH_3$; $R_3 = H$; $R_4 = OH$

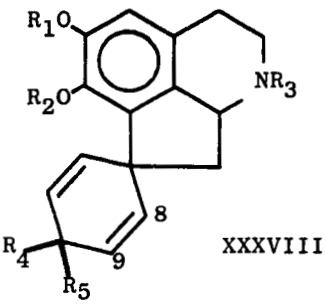
XXXVI: $R_1 + R_2 = CH_2$; $R_3 + R_4 = O$

XXXVII: $R_1 + R_2 = H + CH_3$ or reversed; $R_3 = H$; $R_4 = OH$

The proaporphine alkaloids first reported in 1963 and which bear the general structure XXXVIII, now number at least sixteen, of which fifteen have been covered in a general review.[42] The latest, jacularine (XXXIX) was obtained from Croton linearis Jacq.[43], a source known to yield others in this class. A full paper[44] now records the details of one of the syntheses of this ring system, as exemplified by pronuciferine (XL), by the building up of rings C and D in sequence from a tetrahydroisoquinoline intermediate.

XXXIX: $R_1 = CH_3$; $R_2 = R_3 = H$;
$R_4 + R_5 = O$, and 8-9 reduced

XL: $R_1 = R_2 = R_3 = CH_3$;
$R_4 + R_5 = O$



XXXVIII

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Chapter 30. Advances in Aporphine Chemistry

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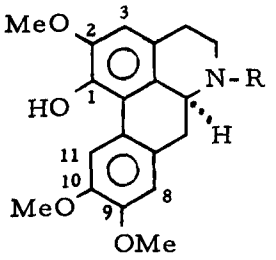
Introduction - Interest in the aporphine alkaloids continues at a rather high level, and a number of developments have been reported since the appearance of the last review (April, 1966)[1] on this class of compounds. More than thirty new aporphines of varied structure have been isolated from natural sources; the structures of a few members have been confirmed by total synthesis. The synthesis of aporphines by methods other than the classical Pschorr method continues to be explored, and some new ring systems related to the aporphines have been synthesized. Some new pharmacological studies employing aporphines have been reported.

This brief review will of necessity be concerned only with selected developments during the period 1967-1968.

New Aporphine Alkaloids - A number of new aporphine alkaloids have been isolated from a variety of plant sources. Structures have been assigned most often on the basis of spectra (especially nmr) and sometimes also by conversion to previously known aporphines. The stereochemistry shown below was not always proven, but is assigned when possible by the reviewers on the apparently valid assumption that all levorotatory aporphines have the D (or R) configuration at the asymmetric carbon atom and that all dextrorotatory aporphines have the L (or S) configuration at this same carbon atom.[2]

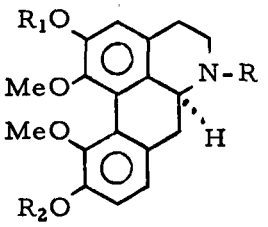
Several aporphines which have been known for some time are included with this group since their structures have only recently been determined or revised. Thus, the structure of leucoxine has been elucidated by an X-ray crystallographic analysis.[3] The structure of hernandine has been revised to the formulation shown below,[4] and the structure of thalicmidine, for many years the subject of controversy, appears now[5] to be that corresponding to the R-enantiomer of dextrorotatory thaliporphine.[7]



Wilsonitrine (R = H)[6]

Thaliporphine (R = Me)[7]

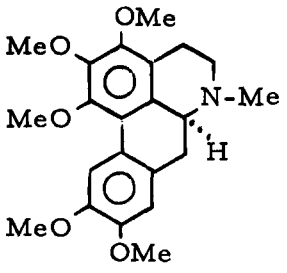
Thalicmidine = (−)-Thaliporphine[5]



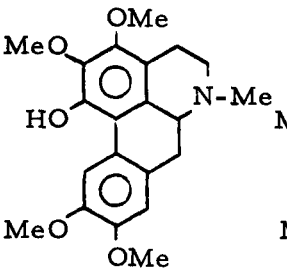
N-Methylhernovine (R = Me, $R_1 = R_2 = H$)[6]

10-O-Methylhernovine (R = R_1 = H, R_2 = Me)[6]

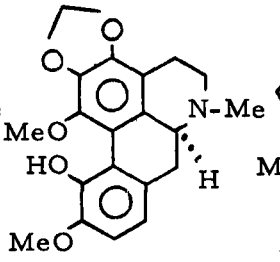
N-Methyl-10-O-methylhernovine (R = R_2 = Me, R_1 = H)[6]



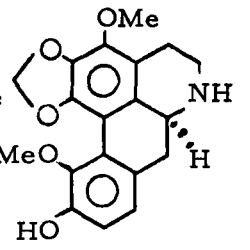
Thalicsimidine[8]



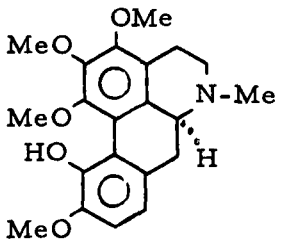
Preocoteine[7]



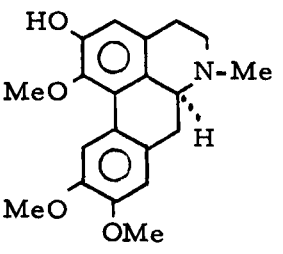
Ocokryptine[9]



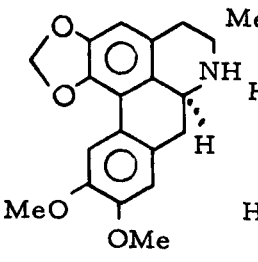
Hernandine[4]



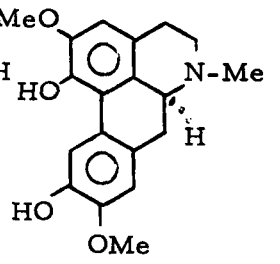
Oconovine[9]



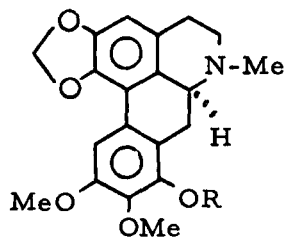
Predicentrine[10]



Nordicentrine[10]

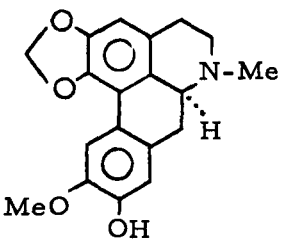


Bracteoline[11]

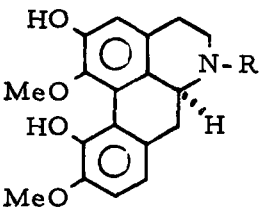


Ocopodine (R = Me)[9]

Leucoxine (R = H)[3,10]

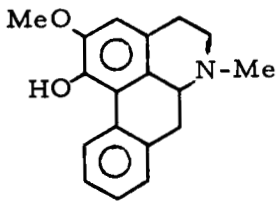


Cassythicine[12]

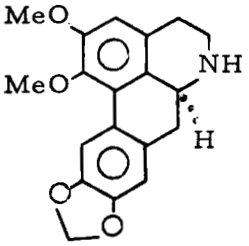


Lindcarpine (R = H)[13]

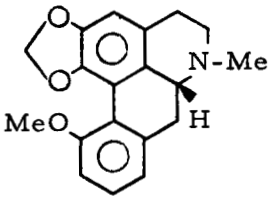
Base from Phoebe clemensii (R = Me)[14]



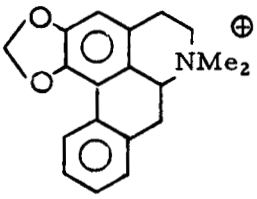
O-Demethylnuciferine[15]



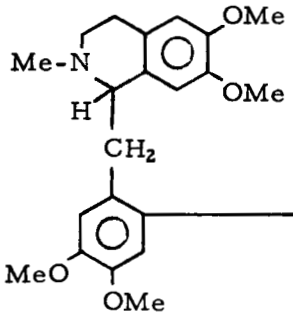
Nornantenine[16]

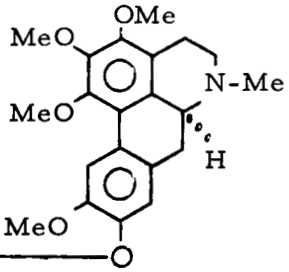


O-Methylpukateine[17]

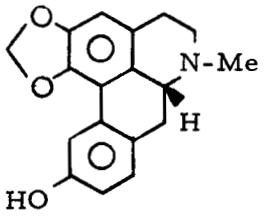


Remrefidine[18]



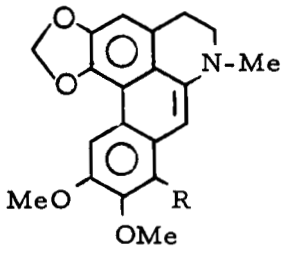


Adiantifoline[19]

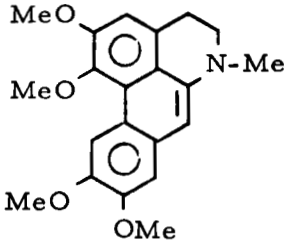


Base from Phoebe clemensii[14]

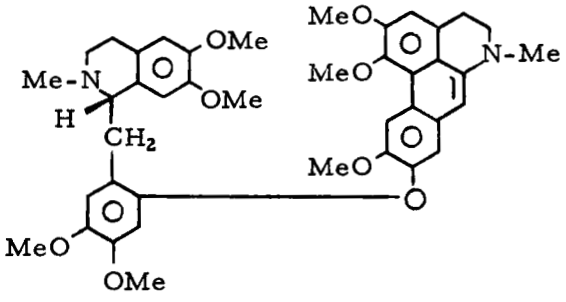
The dehydroaporphines constitute a recently discovered sub-group of aporphine alkaloids. The naturally occurring members found to date are dehydrodicentrine, dehydroocopodine, dehydroglaucine, and the dimeric base dehydrothalicarpine.



Dehydrodicentrine (R = H)[9]
Dehydroocopodine (R = OMe)[20]

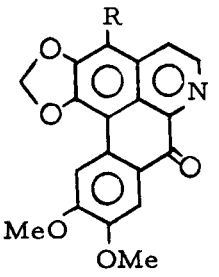


Dehydroglaucine[21]

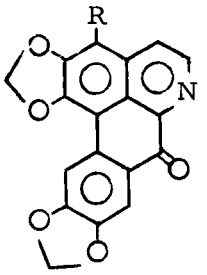


Dehydrothalicarpine[22,23]

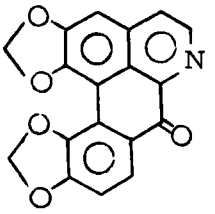
A number of new oxoaporphines have been isolated from natural sources. These are shown below.



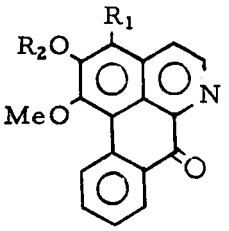
Thalicminine (R = OMe)[24]
Dicentrinone (R = H)[20]



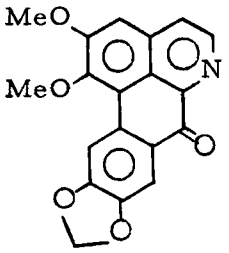
Cassamedine (R = OMe)[25]
Cassameridine (R = H)[25]



Ovigerinone[20]



Moschatoline (R_1 = Me, R_2 = H)[26]
Lysicamine (R_1 = H, R_2 = Me)[27]



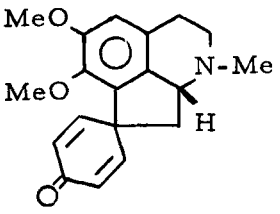
A Base from Cassytha recemosa[16]

Mass Spectrometry - The mass spectra of nine aporphine bases have been recorded and compared. Those alkaloids which are oxygenated at both the 1- and 11-positions give spectra which are significantly different from those of other aporphines.[28] A study of the mass spectra of a number of oxoaporphines has also appeared recently.[26]

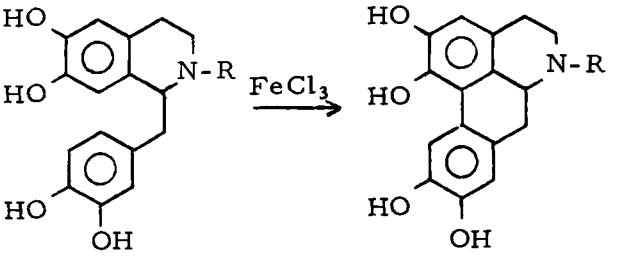
Aporphine Synthesis - The chemistry of the proaporphine alkaloids (typified by pronuciferine) has been reviewed, including their known conversions, both in vivo and in vitro, to aporphines.[29]

The structures of hernandine[30] and of predicentrine[31] have been confirmed by total synthesis using the classical Pschorr route.

Laudanosoline and norlaudanosoline have been converted in good yields into the corresponding aporphines by oxidation with an excess of ferric chloride; cyclic iron complexes are believed to be intermediates in this reaction.[32]



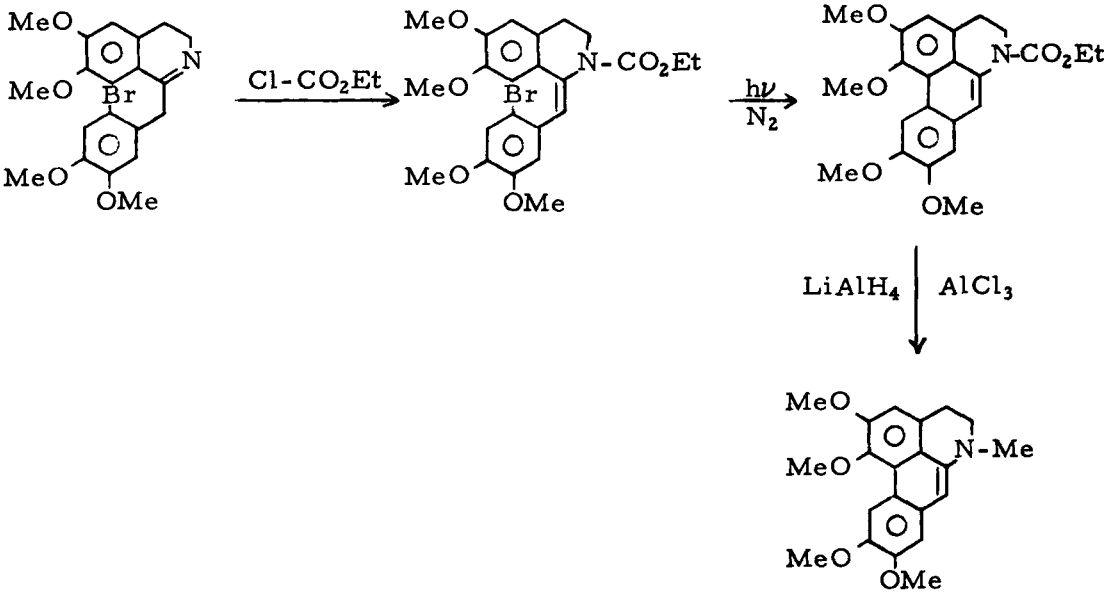
Pronuciferine



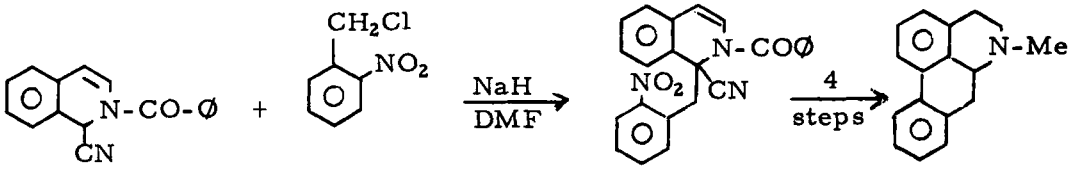
Laudanosoline (R = Me)

Norlaudanosoline (R = H)

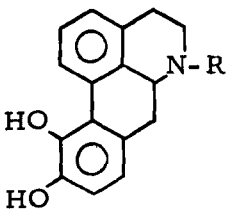
A new non-oxidative photochemical aporphine synthesis has been discovered, in which the key step involves loss of hydrogen halide on photolysis of an appropriately substituted o-halostilbene. In this way, the natural aporphine dehydroglaucine was synthesized as shown below.[33]



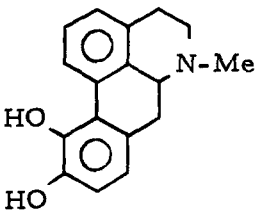
The parent base of the aporphine alkaloids was synthesized in good yield by a new method involving alkylation of a Reissert compound as the key step.[34] The procedure is outlined below.



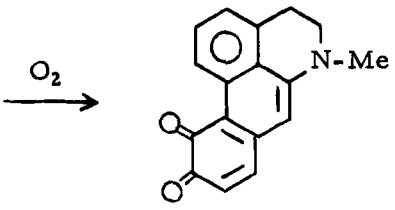
A series of norapomorphine derivatives has been synthesized by the acid-catalyzed rearrangement of the corresponding normorphines.[35] The blue air oxidation product of apomorphine itself has been shown to be an o-quinone of the structure indicated.[36]



N-Norapomorphines (R = H, Et, n-Pr, $CH_2\emptyset$, etc.)

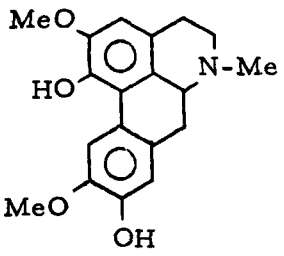


Apomorphine

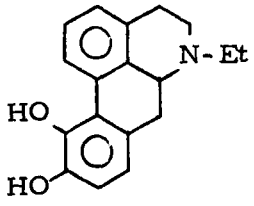


Blue quinone

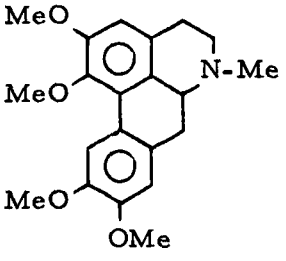
Biological Activity - One of the insect feeding inhibitory constituents of the leaves of Cocculus trilobus DC. has been shown to be the aporphine alkaloid, isoboldine.[37]



Isoboldine



N-Ethylnorapomorphine



Glaucine

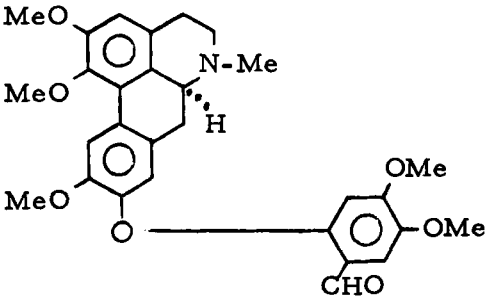
N-Ethylnorapomorphine was found to show emetic stimulant activity in dogs distinctly superior to that of apomorphine; a number of other N-alkylnorapomorphines showed little or no emetic stimulant activity.[35]

Glaucine, bulbocapnine, corydine, and isocorydine all exhibited adrenolytic action in anesthetized cats and rabbits, glaucine having the highest activity.[38] Unlike the other three bases, glaucine showed strong antitussive properties in tolerable doses, and did not cause catalepsy.[38]

Rats, rendered catatonic by bulbocapnine, suffered a loss of about 14% of the normal brain glycogen; adrenol demedullation abolished the hyperglycemic action of bulbocapnine.[39]

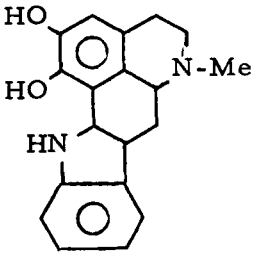
Several of the more complex aporphine alkaloids (thalicarpine, thalmelatine, and hernandaline) showed cytotoxic activity towards monolayer KB cell cultures; dehydrothalicarpine was inactive.[40]

Hernandaline has been found to have appreciable hypotensive activity, as measured in pentobarbitalized dogs.[41]



Hernandaline

Synthetic Aporphine Analogs - The synthesis of two new heterocyclic systems analogous to the aporphine system has been achieved. Thus, the condensed indoline diol I has been prepared,[42] as well as a number of substitution products of pyrido[3,2,1-de]phenanthridine (II).[43]



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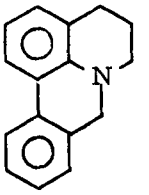

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