# ANNUAL REPORTS IN MEDICINAL CHEMISTRY, 1966

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

*Editor-in-Chief:* **CORNELIUS K. CAIN**
McNEIL LABORATORIES, INC.
FORT WASHINGTON, PA.

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# LIST OF CONTRIBUTORS

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# PREFACE

This is the second volume of an annual series. The aims have not changed from those stated previously: namely, to present a critical summary of new and significant contributions concerning various fields of medicinal chemistry which appeared in the literature during the past year.

Readers familiar with the first volume will notice several changes. We welcome a new section editor and several new authors. New chapters have been added; a few which appeared previously are not represented. Additional changes may be expected in future volumes, both in topics selected for discussion and in viewpoints presented.

All those who contributed to the present volume are busy people who somehow found time to do additional work. Their efforts are deeply appreciated.

Comments of any kind will be most welcome.

Fort Washington, Pennsylvania
June, 1967

Cornelius K. Cain

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## Chapter 1. Antipsychotic and Anti-anxiety Agents

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

Introduction - No new antipsychotic or anti-anxiety drugs were marketed in the United States during 1966 and no development of active chemical structures of major novelty was discernible. Compounds that are chemically new but which belong to well-defined active classes are being studied clinically. An enormous amount of work has been reported on mechanisms of central nervous activity, especially the functions of brain amines.

Biology - Two symposia[1,2] on brain amines have been summarized and another[3] has been published in full. A review[4] on the metabolism of noradrenaline (NA) in the central nervous system has appeared. Krnjević[5] has written a useful review on chemical transmission in the central nervous system.

γ-Aminobutyric acid (GABA) imitates at least qualitatively a cortical inhibitory transmitter.[6] Brain GABA is increased by meprobamate whereas caffeine causes a decrease.[7] These changes are not produced by effects on GABA transaminase or glutamic acid decarboxylase. The anxiety level of dreams has been associated with catecholamine release.[8] Induced emotional stress in rats causes a drop in brain NA but no change in serotonin, GABA or dopamine.[9] Manic-depressive patients in a depressed phase have a lowered urinary output of adrenaline, NA and creatinine.[10] In a manic phase 3,4-dihydroxyphenylalanine (DOPA) output is elevated. Both reserpine and chlorpromazine (CPZ) cause a much greater increase in homovanillic acid (DOPA metabolite) in the corpus striatum of the mouse than does chlordiazepoxide.[11] Blockade of dopamine receptors is believed to be an important factor in the action of neuroleptics.[12]

Timsit[13] observes that the cataleptic effect of the butyrophenones is augmented by parasympathomimetics and hindered by atropine. He hypothesizes that these agents bring about an indirect activation of central cholinergic structures. A cholinomimetic effect of CPZ and related compounds that is antagonized by atropine has been observed.[14] Reserpine and haloperidol, in contrast to CPZ, cause an increase of amine concentrations during the recovery phase of certain cell groups of the lower brain stem, possibly as a compensatory mechanism following blockade of transmission.[15] A good correlation exists between the ability of a drug to counteract the increase of NA in rat brain caused by a monoamine oxidase inhibitor and the reduction of self-stimulation behavior of chronically-implanted rats.[16]

The functions of the brain amines are intimately associated with the continuing discussion of faulty metabolism as a contributing factor in schizophrenia. The entire question has been freshly reviewed by Kety.[17] The "pink spot" as a significant indication of schizophrenia has been attacked[18-20] and its identification with 3,4-dimethoxyphenethylamine has been questioned.[21,22] Wagner, et al.,[23] were unable to demonstrate labelled 3,4-dimethoxyphenethylamine or 3,4-dimethoxyphenylacetic acid after treatment of schizophrenics with labelled DOPA although a normal amount of unlabelled acid was found. An exogenous source of normal amounts of this acid is suggested.[24] It has been shown that 3,4-dimethoxyphenethylamine is not psychogenic in man.[25,26] The metabolism of this agent in the rat leads to the corresponding acid (77%).[27]

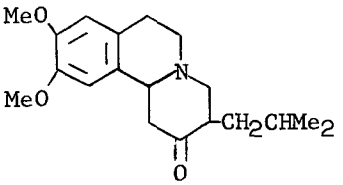
The significance of adrenolutin in schizophrenic urine is still debated,[28] and other unusual indoles in the urine of mentally retarded patients have been detected.[29] A very high rate (10 x normal) of metabolism of ascorbic acid by schizophrenics has led Van der Kamp[30] to administer high doses therapeutically.

Another phase of the search for non-behavioral differences between schizophrenics and non-schizophrenics is concerned with protein fractions. Heath[31] has reviewed his proposal that a protein fraction obtained from schizophrenics and called taraxein may be an antibody to brain tissue and schizophrenia may thus be an auto-immune disease. There is an antibody in human plasma that causes stimulation of chicken erythrocyte glycolysis. Ryan, et al.,[32] were unable to verify a reported quantitative difference between schizophrenic and normal serum in producing this effect. The level of S19 macroglobulins also proved to be uncorrelated with schizophrenia.[33] Conditioned responses in rats were unaffected by injection of schizophrenic serum.[34]

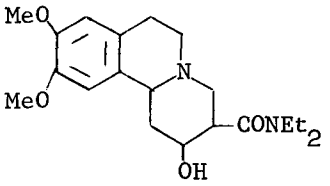
Some reviews on psychopharmacology[35,36] and the problems of drug evaluation in man[37] and animals[38] have appeared. A German symposium[39] on the subject was published. The effect of sixteen psychotropic agents upon food intake was studied in the rat.[40] Phenobarbital, chlordiazepoxide and oxazepam cause increased food intake and a weight gain with the phenothiazines, haloperidol and reserpine causing a weight loss. Avoidance studies in the squirrel monkey show haloperidol almost seven times as potent as CPZ.[41] Tolerance to the effects of thioridazine, chlordiazepoxide and tetrabenazine develop in the conditioned rat.[42]

Amine Depletors - The significance of the observed effects of reserpine and its functional relatives on brain amines is being refined. Rats that have been reserpinized during infancy have a decreased learning ability as adults although the amine depletion is fully dissipated.[43] Reserpine causes an increase in urinary adrenaline and 4-hydroxy-3-methoxymandelic acid but a decrease in NA and DOPA.[44] There is some disagreement about the behavioral effects of prenylamine which lowers brain NA and DOPA to less than 40% of normal values. One group[45] reports muscular weakness as the only gross effect whereas another[46] finds the compound to be a neuroleptic agent. Both prenylamine and tetrabenazine give partial protection

against the behavioral and depleting effects of reserpine indicating competition for sites of action.[47] Benzquinamide, on the contrary, enhances both effects of reserpine. Possibly there are highly specific sites that are affected by reserpine with prenylamine and tetrabenazine affecting only less specific but more numerous sites. The enhancing effect of benzquinamide remains obscure. Benzquinamide is approximately equivalent to CPZ as a tranquillizer[48] and disrupts conditioned behavior at doses that do not decrease brain NA and serotonin.[49] The failure of p-chlorophenylalanine, a selective depletor of serotonin with little effect on catecholamines,[50] to cause sedation suggests that non-specific serotonin depletion is not the mechanism by which reserpine acts.[51] In another view,[52] a high affinity binding of serotonin by rat-brain nerve terminals is associated with its central action and this binding is inhibited by reserpine. The depressive effect of several 5-hydroxytryptophan analogs on motor activity in mice does not appear to be related to any effect on serotonin concentration.[53] Xylopinine[54] is a psychoactive compound that resembles tetrabenazine chemically but does not deplete NA. Ro-4-4602, a decarboxylase inhibitor, has no behavioral effect alone but does augment the behavioral effects of a depletor.[55] α-Methyltyrosine, an inhibitor of tyrosine hydroxylase, produces a depression of conditioned responses that follows a time course similar to that of the observed amine depletion.[56] 10-Acetylyohimbane (W-2045) has a calming effect in dogs and is anti-hallucinogenic.[57]

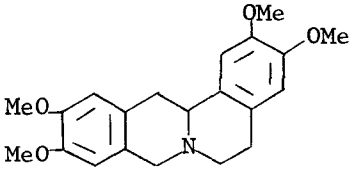



tetrabenazine

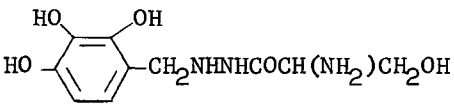



benzquinamide

The metabolism of tetrabenazine proceeds by side-chain hydroxylation, ketone reduction, demethylation of the 9-methoxy group and combinations of the foregoing steps.[58]




xylopinine




Ro-4-4602

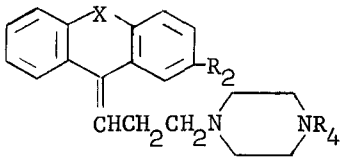
Phenothiazines and analogs - Bradley, et al.,[59] have proposed a neuronal basis for the central action of CPZ: (1) At sites where NA is an excitatory transmitter, CPZ is antagonistic; (2) at inhibitory sites, there is no antagonism, but neuronal depression occurs by other mechanisms such as inhibition of uptake of NA by nerve terminals. The increase of brain phosphatase activity brought about by CPZ may result from the unmasking of normally masked sites of phosphatase action and may have importance in the behavioral effects of CPZ.[60] Elevation of melatonin in the tissues is a

suggested mechanism through which part of the effect of CPZ may be achieved.[61] A study of flavoenzyme inhibition by phenothiazines has been carried out by examination of their effect on D-amino acid oxidase.[62]

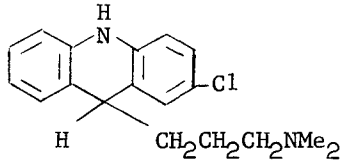
2-Chlorophenothiazine-10-propionic acid has been identified as another metabolite of CPZ.[63] There is no effect of dose on the excretion pattern of CPZ.[64] The principal metabolite of Pasaden (10-azepinylpropyl-2-trifluoromethylphenothiazine) is the 5-oxide.[65]

A discussion of the predictive value of animal tests on phenothiazines has been given by Irwin[66] and a comprehensive review of structure-activity relationships in the tricyclic compounds was published.[67]

Among the new structures attention has been focussed on tricyclic compounds other than phenothiazines although a CPZ analog made from 3-azaspiro[5,5]undecane has been reported to be more potent and much longer acting than CPZ.[68] Potent antipsychotic activity has been observed with clopenthixol[69,70] and thiothixene (P-4657B),[71] two thioxanthene compounds. An acridane analog of CPZ, SKF 14,336, is effective but has side-effects to a degree requiring an anti-Parkinson agent.[72]



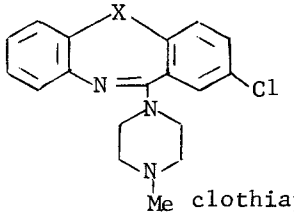

clopenthixol, $R_2$=Cl; $R_4$= $-CH_2CH_2OH$; X=S
thiothixene, $R_2$= $-SO_2NMe_2$; $R_4$=Me; X=S
pinoxepine, $R_2$=Cl; $R_4$= $-CH_2CH_2OH$; X= $-CH_2O-$



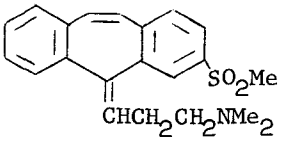

SKF 14,336

Several novel tricyclic compounds have central rings of seven members. Numerous papers[73-75] bespeak a considerable interest in clothiapine (HF-2159) for use in acute psychoses. It is least effective in withdrawn chronic schizophrenics. Although the oxygen analog (LW 3170) of clothiapine is more potent in producing animal catalepsy it is not as useful clinically.[76] Pinoxepine (P-5227)[77] is an antipsychotic agent having marked sedative properties but producing few extrapyramidal symptoms. The antidepressant nortriptyline has a strong sedative component and is an effective tranquillizer.[78]

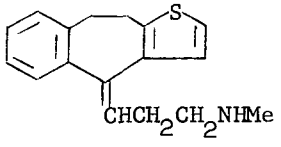
Neither MK-741[79] nor IBD-78[80] give satisfactory results with chronic schizophrenics.




clothiapine, X=S
LW 3170, X=O

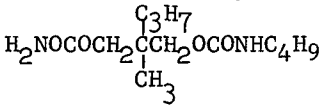



MK-741

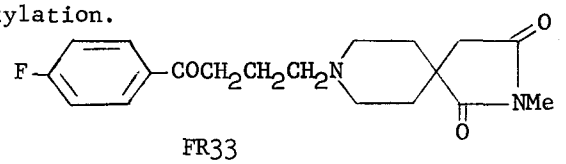



IBD-78

Carbamates - Further studies of the recently marketed tybamate have been reported.[81] Geller[82] has placed it between meprobamate and CPZ in its psychopharmacological effectiveness. Although it attenuates punishment discrimination at higher doses than meprobamate, it blocks avoidance responding at doses that do not block escape responses. The absence of withdrawal symptoms, previously observed in the dog, has been demonstrated in man.[83] Tybamate is metabolized in the dog and rat principally by conversion of its n-propyl substituent into a 2-hydroxypropyl group.[84] This is accompanied by some N-debutylation.



tybamate



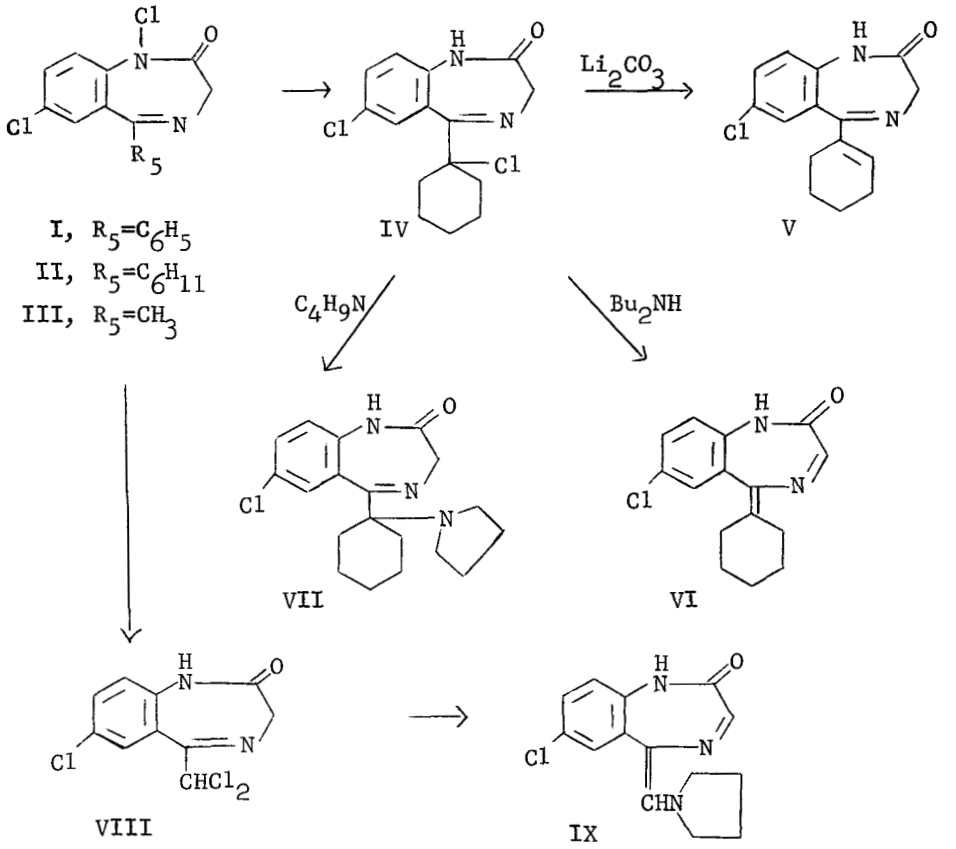
FR33

Butyrophenones - Kreppel[85] has found triperidol to be the most potent of several well-known 4-fluorobutyrophenones in its effect on the central sympathetic tone. Another butyrophenone (FR33) has been recommended for the treatment of stuporous catatonia since it causes no sedation.[86] The 4-p-chlorobenzyl homolog of haloperidol has a more specific effect than haloperidol in the blockade of conditioned avoidance.[87]

Benzodiazepines - A supraspinal site of action has been demonstrated for chlordiazepoxide and diazepam as well as for meprobamate.[88] The anticonvulsant action of chlordiazepoxide resembles that of dilantin instead of that of acetazolamide, being antagonized by reserpine but not by α-methyl DOPA or α-methyltyrosine.[89] This antagonistic effect of reserpine is reduced by α-methyl DOPA, serotonin and amphetamine. The reserpine effect is thus obtained by some means other than its catecholamine-depleting action and the anticonvulsant action of chlordiazepoxide is not mediated by catecholamines.

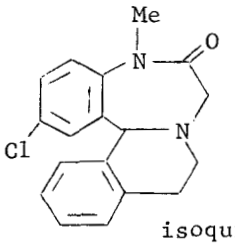
N-Demethylation is a newly described metabolic transformation of chlordiazepoxide in man, the rat and the dog.[90] The effect of dose differences on the observed concentrations of diazepam and its metabolites in the blood and tissues of man has been studied.[91] Repeated 30-mg. doses cause a progressive rise. The principal (demethylated) metabolite appears after 24 hours, rises until its concentration is comparable to diazepam and persists longer than diazepam after dosing ends. Higher chronically administered doses result in a ratio of metabolite to drug of 2.5 to 1.0. Only traces of 1-methyloxazepam and oxazepam are observed in blood. Tissue storage is indicated by the excretion patterns.

Pharmacological and clinical studies have appeared on several unmarketed agents, e.g., prazepam,[92] effective against anxiety, and 7-bromo-1,3-dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one,[93] tested in ambulatory schizophrenics. Schmitt[94] has reported the unusual 1-chloro derivative (I), obtained by hypochlorite treatment, to be a good sedative. The related compound (II) undergoes a rearrangement on heating to give IV which upon dehydrochlorination with lithium carbonate affords the corresponding cyclohexenyl compound (V). The 7-nitro analog of V has muscle-relaxant properties as well as a sedative effect. Treatment of IV with




dibutylamine affords an isomer (VI) of V, whereas treatment with pyrrolidine results in replacement of the chlorine to give VII. It is possible to introduce as many chlorines onto the α-carbon of the 5-substituent as there are initial hydrogen atoms. After introduction of a second chlorine atom onto III to give VIII, treatment with amines leads to such products as IX. The C-chloro compounds are in general less active than the N-chloro analogs as are the compounds having an amine function on the 5-substituent.

Another type of benzodiazepine analog that has been receiving attention arises from the creation of additional fused rings by introduction of an ethylene[95] or methylene[96] bridge between the 4-nitrogen and the adjacent 5-phenyl substituent found in most active benzodiazepines. Isoquinazepon[97] has been described as having a more selective effect than diazepam upon the reticular formation. It potentiates the effect of DOPA in contrast to diazepam. Other analogs have been constructed upon a similar principle by introducing a bond or a

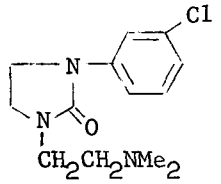



isoquinazepon

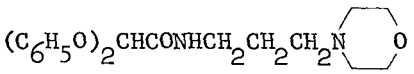
bridge between the 6-position and the adjacent 5-phenyl substituent. Analogs of both chlordiazepoxide[98] and diazepam[99] have been made but test results have not been given.

Miscellaneous compounds - Although lithium salts have been investigated abroad for many years in the control of manic behavior, their study is just beginning in the United States.[100] Lithium ion is effective in phenothiazine-resistant patients but offers little possibility of structural manipulation.[101]

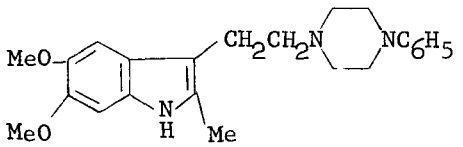
Imidoline is comparable to CPZ in bringing about reduced motor activity but produces much less ataxia.[102] Methionine sulfoximine gives symptomatic improvement in schizophrenics but is somewhat psychotogenic in controls.[103] LL-195 is neuroleptic in the mouse.[104] Phenygam is a strong depressant that produces slowing of the EEG.[105] Extracts of cannabis sativa reduce mouse aggressiveness but are ineffective in reducing the toxicity of amphetamine to grouped mice.[106] Oxypertine, which has been marketed in Europe, is approximately equivalent to CPA in blocking conditioned avoidance but is slightly more toxic.[107]



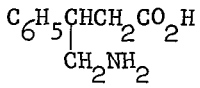
imidoline



LL-195



oxypertine



phenygam

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# Chapter 2. Antidepressants, Stimulants, Hallucinogens

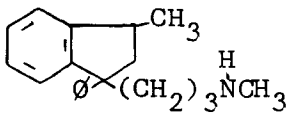
John H. Biel, Aldrich Chemical Co., Milwaukee, Wis.

## I. The Antidepressants

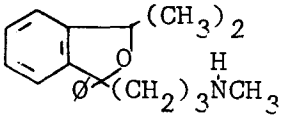
A. Introduction - The major developments in the treatment of depressive illness in 1966 were in (1) the recognition of definable disease entities amenable to selective drug therapy, (2) the wealth of experimental evidence favoring the mobilization of central adrenergic pathways as a workable hypothesis to explain the mechanism of action of the clinically effective antidepressants, (3) more sophisticated chemical structure-clinical activity correlations leading to a much-needed classification of the available antidepressant drugs. This would allow for a more rational approach to the drug treatment of the various depressions and would be based on a careful nosological diagnosis. The latter point is of critical importance in the initial screening of a new agent and its ultimate place in antidepressant drug therapy.

B. The Thymoleptic Agents

1. Newer Structural Entities



(1) Lu 3-057α



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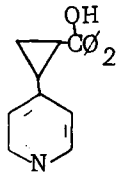
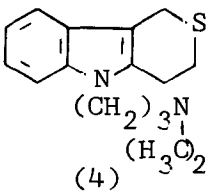
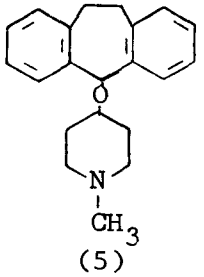


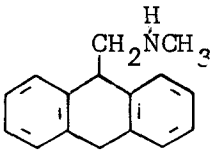
(3) IN 1060
Cyprolidol



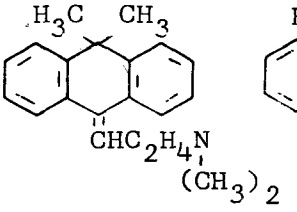
(4)



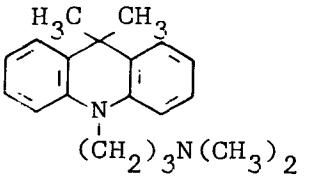
(5)



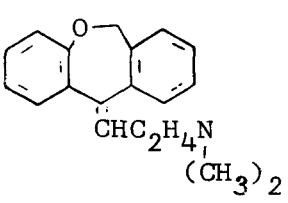
(6) Ciba 30.803Ba



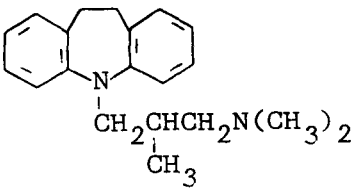
(7) Melitracen
(Trausabun®)



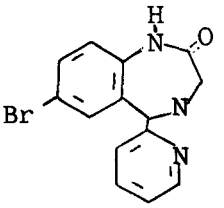
(8) Dimethacrin
(Istonil®)



(9) Doxepin P-4599



(10) Trimipramine (Surmontil®)
Butriptyline (Ayerst)



(11) LA XVII (Roche)

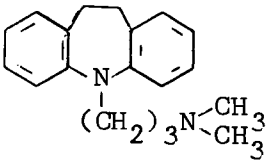
Compounds (1) and (2) were active in mice in reserpine ptosis and norepinephrine (NE) potentiation. Compound (2) had 2 x potency of protriptyline in these tests; it was devoid of anticholinergic, MAO-inhibitory or amphetamine-like activities[1-3]. The pharmacologic spectrum of cyprolidol (3) was similar to that of imipramine except that it blocked the tyramine-induced rise in blood pressure only in anesthetized dogs but potentiated it in conscious dogs[4-7]. In man, cyprolidol was less effective than imipramine[7]. Structures (4) and (5) displayed moderate antireserpine activity in mice[8,9]. Compound (6) antagonized the central stimulant effects of caffein and mescaline and inhibited spontaneous aggression in mice. Its antireserpine effect was less than that of imipramine. In functional depressions, the drug was quite effective and rapid-acting (3-6 days)[10]. Melitracen (7) was a sedative-type antidepressant in man similar to amitriptyline. The drug was anticholinergic and produced favorable effects in 25 of 38 patients and confusional states in 2 of 38 individuals[10-11b]. Dimethacrin (8) has been evaluated quite extensively both in animals[12,13] and man[14-16]. In clinical efficacy and spectrum of side effects, the drug was said to be comparable to amitriptyline and imipramine, but superior to protriptyline. A high success rate was obtained in anxiety depressions[16]. Dimethacrin was synthesized originally by Molnar and Wagner-Jauregg[17]. Doxepine (9), a centrally active anticholinergic, antagonized the hypothermic effects of reserpine and fighting behavior in mice[18]. Clinically, the compound was not as effective as other tricyclic antidepressants in the therapy of endogenous depressions. Because of its high incidence of sedation, it was more useful in the anxious and agitated depressed patient[19-21]. Trimipramine (Surmontil®) is best characterized as a neuroleptic antidepressant. It displayed good efficacy in psychotic, anxious and endogenous depressions and hence, had a greater therapeutic spectrum than imipramine[22-25]. A pyridine congener of diazepam (11) exhibited both anti-anxiety, as well as mood-elevating properties in chronic psychotic patients[26].

2. Mechanism of Action - Increased strides were made toward arriving at a biochemical basis for endogenous depression. The argument runs somewhat as follows: reserpine causes a depression in 15% of the patients which cannot be distinguished from a true endogenous depression. Biochemically, this "model" depression is accompanied by a 50% drop in the urinary excretion of amine metabolites indicating that the neurotransmitter amines are released intracellularly by reserpine and metabolized intracellularly by MAO without ever exerting a physiologic effect at the adrenergic nerve endings[27]. During treatment with imipramine, urinary normetanephrine increased and vanillylmandelic acid (VMA) levels decreased starting at the time of the period of improvement. The decreased VMA excretion is thought to be due to the decreased intracellular degradation of norepinephrine (NE) by MAO brought about by the ability of the antidepressant drugs in preventing the re-uptake of "active" (extracellular) NE at the cell membrane into intracellular storage. The re-uptake of physiologically active NE by the cell represents one of the main pathways of NE inactivation[28-31]. Extracellular ("active") NE is metabolized by catechol O-methyltransferase (COMT) to normetanephrine at the adrenergic synapse. Schanberg et al.[32] demonstrated that imipramine and desipramine, but not chlorpromazine (CPZ), slowed the disappearance of previously administered $H^3$-normetanephrine. Additional evidence is provided by the classical experiments of Murad and Shore[33] which showed that pretreatment of rats with four tricyclic antidepressants greatly potentiated the ability of tetrabenazine to release metaraminol-$^3H$ from adrenergic stores in heart and brain. Metaraminol is not metabolized by either MAO or COMT, but resembles NE with regard to storage, release and re-uptake by the adrenergic cells. Glowinski et al.[34] studied the effects of desmethylimipramine, pheniprazine and amphetamine on the disposition and metabolism of $H^3$-NE and $H^3$-dopamine in various regions of the rat brain. Deaminated metabolite levels were "severly reduced," while $H^3$-normetanephrine levels were "strikingly elevated." Meisch et al.[35] demonstrated that desipramine, but not reserpine, can block the β-hydroxylation of $^3H$-α-methyldopamine and $^3H$-α-methyltyramine suggesting that hydroxylation takes place inside the cell membrane, but not in the reserpine-sensitive storage sites.

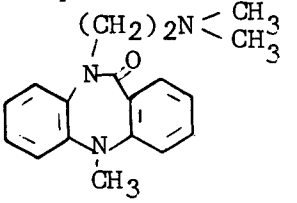
3. Structure-Activity Relationships - An excellent and most comprehensive treatise concerning the correlation of chemical constitution and clinical efficacy of psycho-active drugs has been published by Stach and Pöldinger[36]. In regard to the tricyclic antidepressant drugs, the "skewed" character of the fused ring system, the effect of ring substituents, the nature of the aminoalkyl side chain, as well as the size of the middle ring are discussed in relation to the potency and type of action of the resulting antidepressant drugs. Pöldinger[37] has classified the currently available tricyclic drugs

according to their clinical spectra and chemical structures:

I. Mood-elevating

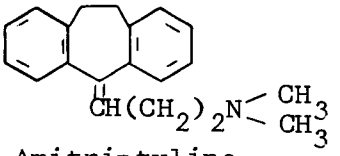


Imipramine

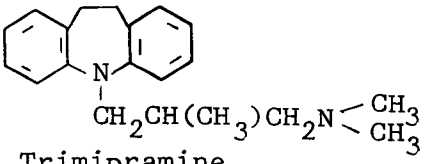


Dibenzepine (Noveril®)

II. Anti-anxiety and Anti-agitation Properties in Antidepressants

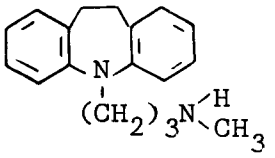


Amitriptyline

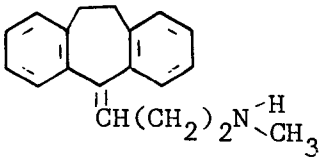


Trimipramine

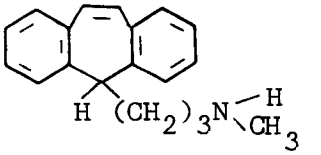
III. Stimulant Antidepressants - The structures shown below, as well as the MAO inhibitors are used in the inhibited, withdrawn (anergic) depressed patients. They also have a greater tendency to exacerbate psychotic symptoms:



Desipramine

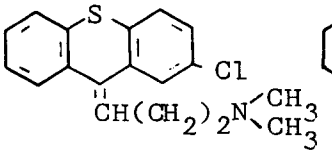


Nortriptyline

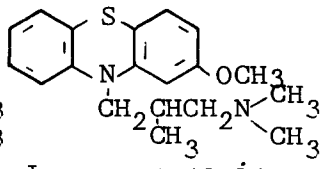


Protriptyline

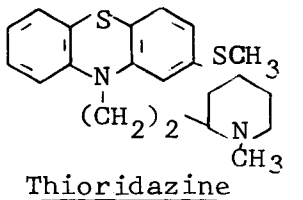
IV. Neuroleptics with Mood-elevating Properties



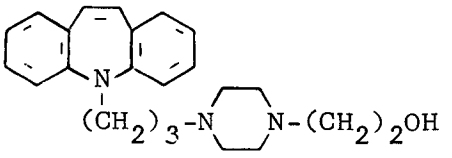
Chlorprothixene



Levomepromazine



Thioridazine



Opipramol

C. Clinical Considerations - One of the most vexing problems in the past has been how to recognize potential antidepressant activity of a new drug in animals, especially when such a disease entity is presumably non-existent in the animal kingdom and mental depression itself is such a mosaic disease syndrome in man. Pöldinger[37] has laid down certain pharmacologic criteria which the presently available thymoleptics have fulfilled and which distinguish them from the neuroleptics, central stimulants, and MAO inhibitors:

| | Stimulants | MAO-I's | Thymoleptics | Neurolept. |
|---|---|---|---|---|
| Reserpine Antagonism | functional | biochem. | functional | none |
| Catalepsy | anticatal. | anticatal. | anticatal. | catal. |
| Principal Auton. Effects | potentiate adren. Functions | ---- | anticholin. | adrenolytic |
| Effects on Reticul.Form'n | stimul. | stimul. | stimul. | inhib. |

Obviously, this spectrum of pharmacologic activities has been derived post facto from the properties of the tricyclic antidepressants and it will be interesting to see whether it will be useful in predicting clinical antidepressant activity of totally unrelated structures.

Another problem which has beset the clinical investigator has been the confusion as to what constitutes a primary clinical depression. A recently published paper by Miller[38] goes far in laying down guidelines for the diagnosis of endogenous depression and is "must" reading for anyone working in the area of mental depression. The main points Miller makes are the following:

> (1) Endogenous depression is a definite clinical entity of organic (pathophysiologic) origin. (2) It affects a stable subject with a previously good personality history, whereas reactive depression affects the unstable and inadequate personality. (3) Endogenous depression strikes "out of the blue" with no apparent environmental causes, although endocrine changes, prolonged viral infections, hepatitis and mononucleosis may at times precede it. (4) It is characterized by early morning awakening and progressive improvement as the day goes on, as well as by constipation, loss of both appetite and sexual interest. (5) Endogenous depression responds readily (in 75% of the cases) to ECT and drug therapy, whereas reactive depressions are often resistant to drug therapy. It is "a physical illness treatable by physical means". Both Pöldinger[19] and Hollister et al.[39]

point to the crucial importance of nosological diagnosis in the evaluation of a new antidepressant, since only the improvement in endogenous and involutional depressions is the criterion for classifying a new drug as an antidepressant. Chlorpromazine is active in reactive and schizophrenic depressions, but inactive in endogenous depressions.

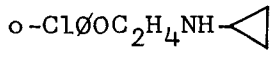
5. Combination Therapy - The combination of amitriptyline and perphenazine (Triavil®, Etrafon®) has been applied primarily in the therapy of reactive, anxiety and psychotic depressions. While most reports were favorable, Hollister et al.[41] could not confirm the superiority of the combination over amitriptyline alone in retarded or anxious depressions. The addition of an MAO inhibitor (pheniprazine) to a chlorpromazine regimen in chronic withdrawn schizophrenics resulted in significant improvement leading to a discharge of 60% of the patients who had been hospitalized for many years on just tranquilizer therapy alone[42]. Sargant et al.[42] report on a series of 73 patients referred to the authors for leucotomy because of intractable chronic tension. The addition of iproniazid to the previously unsuccessful regimen of amitriptyline, narcosis and ECT necessitated leucotomy in only 18 of the 73 patients.

6. Side Effects of Antidepressant Drugs - Recent publications pertaining to the clinical side effects of antidepressant agents are by Hollister[44], Kahr et al.[45], Tschen et al.[46], Blair and Simpson[47] and Simpson et al.[48]. The toxicology of amitriptyline has been described by Myers et al.[49].

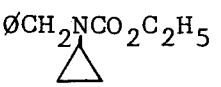
7. Imipramine Metabolites - The desmethyl- (DMI) and the 2-hydroxyimipramine are the major metabolites of imipramine. In addition, sixteen others have been detected in rat liver microsomes[50]. Theobald et al.[51] have reported on the central and peripheral effects of six imipramine metabolites in several animal species.

C. MAO Inhibitors

1. Newer Structures



(12) (Lilly 5164)



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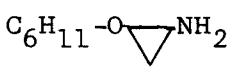

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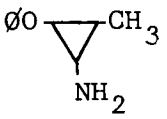

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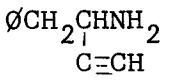
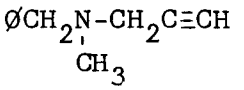
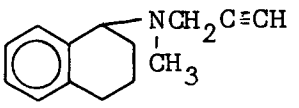


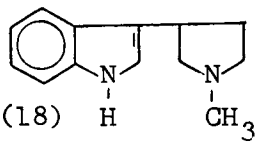
(16)



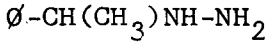
Pargyline



(17)



(18)



(19) Mebanazine (I.C.I.)

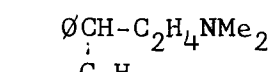
Compound (12), a potent MAO inhibitor, caused schizophrenic patients to become overactive, irritable, more psychotic and aggressive. These effects are characteristic of most stimulant-type antidepressants, including the thymoleptic drugs. Paradoxically, this compound also produced sedation and an increase in sleep[52]. Compound (13) proved to be effective in a small number of newly admitted depressed patients. Although less efficacious than amitriptyline, it appeared to have a lower incidence of side effects[53]. While (14) produced MAO inhibition in animals comparable to the phenyl derivative and tranylcypromine (Parnate®), the addition of a methyl group in structure (15) abolished activity[54,55]. Shifting of the N-propargyl group in pargyline into the alkylene side chain produced a compound (16) equal to pargyline in unmasking the CNS stimulant effects of β-phenethylamine in mice. However, this agent was much shorter-acting which would tend to indicate a reversible-type of MAO inhibition. As an overt CNS stimulant, the drug had 1/25 the activity of amphetamine[56]. The tetrahydronaphthyl analog of pargyline (17) had 20 times the activity of the latter in mice[57]. A "cyclized" etryptamine (Monase®) derivative (18) produced reserpine reversal and MAO-I in mice at 50 mg/kg[58]. Mebanazine (Actomol®) (19) in animals is claimed to be a more potent MAO-I than pheniprazine with a greater therapeutic index[59]. The drug appeared to be "devoid of specific antidepressant effects" in man, but at daily doses of 30 mg it precipitated a "severe and prolonged hypotensive collapse" in 3 of 7 patients. In diabetics on insulin therapy, the drug caused a spontaneous hypoglycemic response[60]. The addition of an MAO-I to a neuroleptic drug regimen in anergic withdrawn psychotics was discussed in the previous section.

The "False Neurotransmitter" concept as a basis for explaining the mechanism of the hypotensive action of MAO-I's is presented in greater detail by Cohen et al.[61]. The delayed action of the MAO-I's with respect to their clinical antidepressant and hypotensive activities is presumably due to an indirect effect dependent on the gradual accumulation of monoamines at the adrenergic nerve fibres. According to the

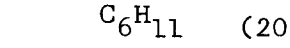
experiments of Carlsson and Waldeck[62], $^3$H-metaraminol is released only slowly by the MAO-I's from the nerve fibres. This effect can be blocked by decarboxylase inhibitors which prevent monoamine formation.

II. Central Stimulants

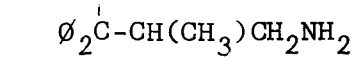
A. New Structures



(Win 1344)



(20) Gamfexine



(21) (U-23,807A)

Win 1344 (20) was capable of stimulating depressed patients. In combination with thioridazine, it worked well in withdrawn schizophrenics[13]. Gershon et al.[64] found the compound less efficacious than imipramine (37 vs 54%). The stimulant properties of the drug often resulted in exacerbation of psychotic symptoms. Compound (21) had 1/3 the activity of amphetamine in increasing spontaneous motor activity. The effects of single oral doses in cats and dogs were still present after 24 hours. Unlike amphetamine, U-23,807A lowered body T$^o$ and potentiated hexobarbital and ethanol narcosis at two and four times the dose of chlorpromazine, respectively[65]. The branching of the alkylene side chain virtually eliminated all peripheral anticholinergic effects. The synthesis of this series has been described by Moffett et al.[66] and the pharmacology by Keasling et al.[67]. The *l*-isomer is now undergoing clinical trial.

Magnesium pemoline was briefly alluded to in the 1965 "Annual Reports of Medicinal Chemistry" (page 23) as promoting the biosynthesis of RNA in rat brain and facilitating learning by increasing memory and retention of learned behavior. Subsequent reports have made these findings controversial and the reader is referred to the cited references[68-72] for further guidance on this subject.

The mechanism of the central action of amphetamine continues to be explored and the prevailing evidence points to multiple mechanisms involving catecholamine release, inhibition of catecholamine uptake, MAO inhibition and a direct intrinsic action[73-75]. Dependence on amphetamine and other stimulant drugs, its clinical manifestations and treatment are the subject of two excellent reviews[76,77].

III. The Hallucinogens

A. Psychedelic Therapy - The increased use of psychedelic ("mind expanding") drugs in personality disorders probably represents one of the more important developments in this area. Thus, Leuner[78] found these drugs an "excellent adjuvant" in the psychotherapy of character neuroses, psychopathic

personalities, neurotic depressions, anxiety neuroses and hysteric conversion symptoms which did not yield to any other form of treatment. Remissions were obtained in 76% of the cases. Malitz[79] found 0.07 mg of LSD equivalent to 500 mg of mescaline in facilitating transference during psychotherapy. Alcoholism was particularly amenable to this type of therapy. Savage et al.[80] obtained similar results as the two previous investigators in patients demonstrably low in motivation, socio-economic status and educational level. Variability in response was due to personality, previous history of the patient, and the particular therapeutic environment. Bender[81] found it easier to break through the autistic (self-centered) behavior of schizophrenic children with the use of LSD. Abramson[82] has reviewed the application of LSD, psilocybin, peyote, marijuana in psychotherapy. The dangers of LSD ingestion in a non-therapeutic environment have been summarized by Cohen[83] and Fink[84]. Emotionally labile persons were particularly susceptible to the psychotogenic properties of the drug. The phenothiazine tranquilizers minimized such risks, if administered as soon as the acute LSD syndrome had been observed[84].

B. Endogenous Hallucinogens - The subject of abnormal amine metabolism in schizophrenics was reviewed in the 1965 "Annual Reports of Medicinal Chemistry" (page 25). Hollister and Friedhoff[85] were unable to produce any effects in volunteers with DMPEA (3,4-dimethoxyphenethylamine), whereas mescaline in similar doses produced profound effects of depersonalization and perceptual distortion. DMPEA is metabolized mainly to 3,4-dimethoxyphenylacetic acid, whereas mescaline is excreted largely unchanged[86]. Heller[87] found that the administration of MAO-I's to schizophrenics exacerbated their psychotic symptoms and produced excretion of a "bufotenin-like" substance. Other investigators have been unable to confirm such findings of abnormal amine metabolism in schizophrenics[88-90].

C. Anticholinergic Hallucinogens (Clinical Effects) - Neubauer et al.[91] claim that antagonism to Ditran may require both adrenolytic and cholinomimetic activities. Bauer[92] and Flügel[93] were able to inhibit the psychotomimetic effects of Bayer 1443 and Ditran by the prior administration dihydroergotamine. Increased urinary excretion of catecholamine and serotonin metabolites after the administration of N-methyl-3-pyrrolidyl phenylcyclopentylglycolate was demonstrated by Bente et al.[94].

D. Pharmacologic and Biochemical Effects - The finding by Brown et al.[95] that the acute administration of chlorpromazine (CPZ) and Ditran produced a deep coma in dogs and that the substitution of CPZ by imipramine elicited a much milder response might possibly be exploited as a screening test for novel psychotropic drugs.

In an effort to elucidate the mechanism of action of psychotomimetic glycolate esters, Rogeners et al.[96] studied the action of Ditran on the surface pressure and viscosity of lecithin mono-layers. Their findings indicate that Ditran might be associating with the phosphate group and the fatty acid chains of lecithin. The drug forms associations with calcium and phosphate ions and with ATP.

Votava et al.[97] found no pharmacological or behavioral differences in animals with LSD and a number of its congeners. In man, only LSD caused psychic disturbances. On a biochemical basis, a distinction could also be made in that only LSD caused a rise in serotonin plasma levels.

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

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Introduction: During 1966, several new types of chemical structures were reported to have central nervous system depressant activity, and further studies were described of compounds previously classified in this area. The problem of defining further the type of activity to predict whether a given depressant will be of clinical application as an anti-psychotic, anti-anxiety agent, sedative, hypnotic, anti-convulsant or anesthetic continues to occupy the attention of many investigators. Several approaches to the problem have been used.

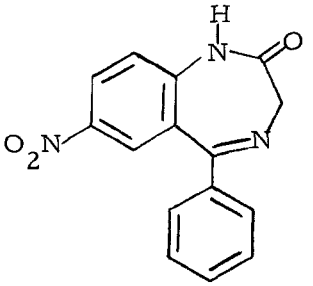
Krnjevic and Videk[1] described a new screening test based on a "natural" reaction of a rat, namely the entrance into a darkened cage through a hole of suitable size (5 cm.). Either of several depressants, chlorpromazine, reserpine, phenobarbital, etc. increased the time required for this reaction. A pyschostimulant, amphetamine, decreased the time, as did the anti-depressants imipramine or nialamide.

The effect of administration of various compounds to animals or man on the formation, metabolism and concentration in organs or tissues of biogenic amines continues to be actively pursued in many laboratories. Of particular interest are recent studies on serotonin. Brodie and co-workers[2] suggested that the sedative effects of reserpine are related to the initial rate at which serotonin is released from the brain. Koe and Weissman[3] found that p-chlorophenylalanine is a potent and selective depletor of brain serotonin in mice, rats and dogs. No behavioral changes were noted in rats even when the serotonin level was reduced to less than ten percent of the control value; however, the characteristic signs elicited by reserpine or tetrabenazine in these animals were not blocked. Cremata and Koe[4] reported clinical pharmacological evaluation of p-chlorophenylalanine in six normal humans. Gradual increase in dosage to 3 grams per day resulted in a decline in blood serotonin to 60-70% of pretreatment levels and in urinary excretion of 5-hydroxy-3-indoleacetic acid to 10-50%. No clear-cut signs of effects on the central nervous system were noted.

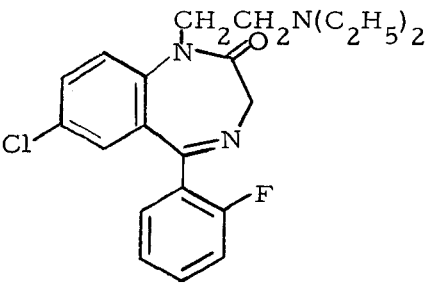
References may be found in the literature to the influence of almost every known CNS depressant on the EEG pattern. Eidelberg, Miller and Long[5] report effects of several psychoactive compounds, especially

those altering biogenic amine levels, on EEG's in cats; they include many references. There is growing interest in the effect of CNS depressants, particularly hypnotics, on sleep patterns. Excellent introductions to the subject may be found in the proceedings of a symposium[6] and in a review by Pieri and Hürlimann.[7] A more recent symposium on the same subject was held in Würzburg in 1966. Proceedings should be published shortly.

Sedatives and Hypnotics: Additional reports[8, 9, 10, 11, 12, 13] have appeared describing successful clinical use of nitrazepam, Mogadon®, RO 4-5360 (I) as a hypnotic in doses of 5 or 10 mg., especially in older patients. However, some "hangover" occurred, particularly with the 10 mg. dose.



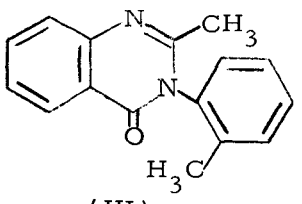
(I)



(II)

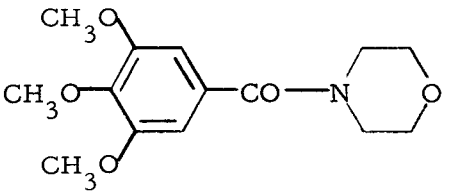
A related compound, RO 5-6901 (II), was studied in 24 patients and found to be as effective a hypnotic in a 15 mg. dose as was 100 mg. of secobarbital.[14] The effect of nitrazepam on sleep patterns in humans was compared with that of phenobarbital and diazepam.[15] Nitrazepam differs from the other drugs in the pattern of alpha and beta waves in deeper sleep patterns; this may be indicative of paradoxical sleep.

Methaqualone (III) was reported to be as effective in a 200 mg. dose as 100 mg. secobarbital without significant side effects[16] and to show no evidence of hepatic dysfunction.[17] A study of the metabolism of methaqualone in humans[18, 19] resulted in the isolation from the urine of six metabolites resulting from hydroxylation of the 2-methyl group, the o-tolyl group or the benz portion of the nucleus.



(III)

Trioxazine (Trimetozine) (IV) was described as an effective daytime sedative[20] in a dose of 300-1500 mg. per day and as a mild tranquilizing drug which gave a false positive urine glucose test in two patients using enzyme test papers.[21] The generic name Roletamide was registered for CL 59,112 (V) as a hypnotic;[22] no further published information could be found.



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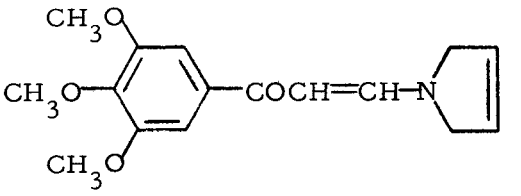
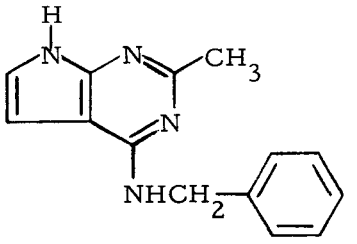


(V)

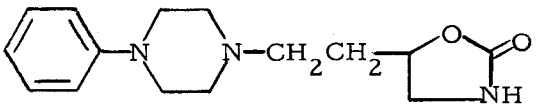
Sjoqvist and Lasagna[23] reported that 25 or 50 mg. of doxylamine (Decapryn®) succinate was more effective clinically as a hypnotic than 100 mg. of secobarbital but not as effective as 200 mg. of the latter. They caution that no suitable antidote is available in case of over-dosage.



(VI)

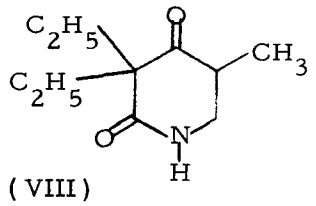
In a detailed study of a pyrrolopyrimidine, BW 58-271 (VI), Norton and Jewett[24] found that the compound was a potent hypnotic agent (three times as active as hexobarbital). It also had local anesthetic and hypotensive activity. Its most unusual effect was sudden suppression of the EEG in conscious cats lasting for several minutes after intravenous injection.

DaVanzo and co-workers[25] reported that an oxazolidinone derivative, AHR 1209 (VII), showed psychosedative activity in animals.



(VII)

Three metabolic products of methyprylon (VIII) were isolated and identified ten years ago.[26] Bösche and Schmidt[27] recently identified a 3-oxoglutarimide (IX) as an additional metabolite isolated from human urine.



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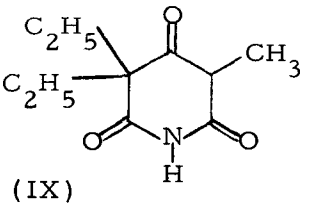
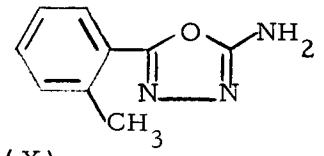


(IX)

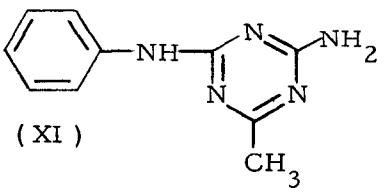
Central Muscle Relaxants: Chlorophenesin carbamate (Maolate®) was first marketed in 1966. Its pharmacology was thoroughly described by Matthews and co-workers[28] in 1963. Several clinical studies have been reported.[29, 30, 31] Metabolic studies[32, 33, 34] showed that in man, about 85% of the administered dose was excreted in the urine as the O-glucuronide. Similar results were obtained in the rat. In the dog, the major metabolite was the same compound, but appreciable amounts of acidic products resulting from oxidation to p-chlorophenoxylactic acid, p-chlorophenoxyacetic acid and p-chlorophenol were also isolated.



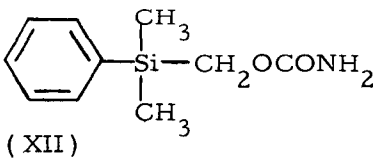
(X)

Yale and Losee[35] prepared forty-six 1, 3, 4-oxadiazoles and oxadiazolines and found many of them to be moderately active muscle relaxants. The most potent of the series was the compound of structure (X).

Brittain[36] reported that a triazine derivative, CB 2487 (XI), resembled mephenesin pharmacologically, both qualitatively and quantitatively.



(XI)



(XII)

A compound of unusual structure (XII), a silacarbamate, was prepared by Fessenden and Coon[37] and found to show muscle relaxant activity of brief duration.

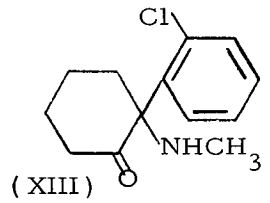
Anti-convulsants: Screening for this type of pharmacological action continues, and active compounds are found although none appear to be promising enough to warrant further investigation. Swinyard and Castellion[38] studied the anti-convulsant properties of a series of benzodiazepines and suggested that a compound offering appreciable advantages over those currently in use as anti-epileptics might be found in this area.

Mishie, Weary and Berger[39] reported that a series of chemically related thiosemicarbazide, thiourea or urea derivatives may be divided into groups of convulsants, anti-convulsants and those of mixed action.

Anesthetics: Two excellent reviews have recently appeared. Dobkin and Su[40] discussed all types of new general anesthetics (including electrical) from the viewpoint of clinical utility. The various agents were compared with respect to induction, emergence, hypnosis, analgesia, etc., and side effects were listed. Desirable properties of an improved anesthetic were included. Dundee[41] reviewed inhalants, barbiturates and propanidid from the viewpoint of clinical pharmacology.

(a) Inhalants: A summary of the National Halothane Study (Bunker Committee Report) was published.[42] Data from more than 850,000 anesthetic administrations were compiled and studied. Halothane deaths amounted to 1.87% compared to 1.93% for all anesthetic practices. The possibility could not be excluded that there may be a halothane-related hepatic necrosis, but if so, it occurs very rarely. The study demonstrated that the overall death-rate problem is much greater in its implications for patient care than the problem of massive hepatic necrosis.

(b) Injection Anesthetics: Corssen and Domino[43] supplemented their previous study of CI 581 (XIII), an analog of phencyclidine (Sernyl®) (XIV), with a report of 139 clinical cases in which adequate anesthesia



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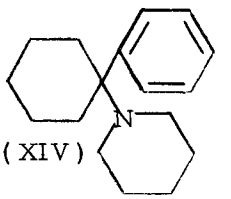


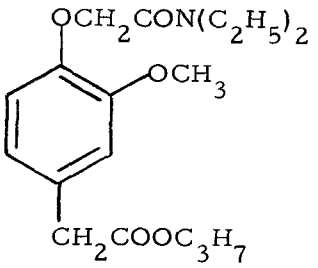
(XIV)

was obtained in 92%. Emergence was occasionally associated with a psychotomimetic response which was controlled by small doses of a barbiturate.

Interest in γ-hydroxybutyric acid (or its sodium salt or the lactone) as an intravenous anesthetic[40] continues. There appears to be some question whether the compound has sedative and hypnotic properties in low doses. Metcalf, Emde and Stripe[44] studied EEG and behavioral properties of the compound in humans and reported that there was a sudden shift from responsivity to unconsciousness. On the basis of the EEG records, they speculate that the drug depresses reticular centers which ordinarily inhibit the onset of slow sleep.

Gessa and co-workers[45] reported that intraperitoneal administration of γ-hydroxybutyric acid to rats and rabbits produces marked and rapid increase in brain dopamine which was not due to monoamine oxidase inhibition or to stimulation of dopamine synthesis. No appreciable change in brain norepinephrine or serotonin occurred. The loss of righting reflex which was observed correlated temporally with high dopamine levels in the brain.

Sprince, Josephs and Wilpizeski[46] measured the effects of γ-hydroxybutyric acid and several related compounds by noting loss of righting reflex after intraperitoneal injection in rats. γ-Butyrolactone was active more quickly and for a longer time than equivalent amounts of the acid. 1, 4-Butanediol was comparable in activity to the acid, but 1, 3-butanediol was much less active. Pyruvate prevented or reversed the LRR effect of the acid, the lactone and the 1, 4-diol.

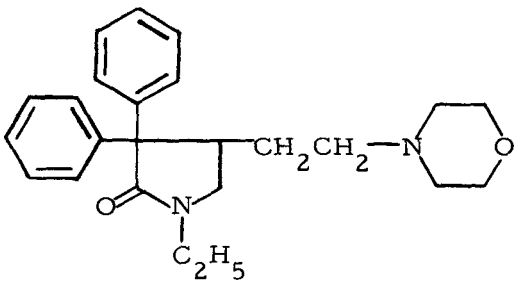



(XV)

Several articles indicate that there is continuing interest in propanidid (XV) as an ultra-short anesthetic. Boureau[47] and Dumoulin et al.[48] reported favorable results in minor procedures in ambulatory patients, as an induction anesthetic and in convulsive therapy. Swerdlow[49] considered it worthy of further investigation in dental anesthesia. Clarke and Dundee[50] included their considerable number of publications on propanidid anesthesia in a survey of experimental and clinical pharmacology of the compound.

Adjuncts to Anesthesia

(a) Respiratory Stimulants: Doxapram (XVI) appears to offer advantages over other analeptics for this action, but it does not appear to be widely used clinically. Klemm[51, 52] found that it increased respiratory minute volume as much as 200% in one minute in dogs deeply anesthetized with sodium pentothal, an effect much greater than that using the best combination analeptics reported by others.



(XVI)

Funderburk, Oliver and Ward[53] reported that intravenous administration of doxapram to cats and dogs caused selective stimulation of the respiratory centers in the medulla in low doses (0.2-1.0 mg./kg.), stimulation of the cord in higher doses (3 mg./kg.) and stimulation of higher centers in the brain in still larger doses.

(b) Neuroleptanalgesics: Brief reviews of this topic may be found in reference 40 and in a chapter by Corssen.[54] More information is contained in two symposia on the subject which have been recently published, one held in Edinburgh in 1964[55] and another in Zurich in 1965.[56]

By far the most widely used preparation of this type is Innovar®, a combination of the neuroleptic, droperidol, and the analgesic, fentanyl. Some 30 articles appeared during 1966 describing its use in humans with or without nitrous oxide.

Innovar® Vet continues to find wide application for surgical procedures in dogs. A recent article[57] indicates equally good results in primates.

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# Chapter 4. Analgetics --- Strong and Weak

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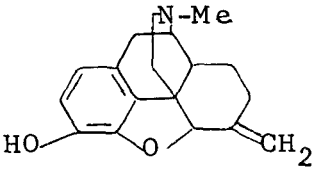
University of North Carolina, Chapel Hill, N.C.

I. Introduction - The past year in the field of analgesics must be considered one of consolidation and expansion. There were no startling breakthroughs in our understanding of analgesics nor were any markedly different new classes of analgesics discovered.

## II. Strong Analgesics

A. Morphines and Morphinans - Abdul-Rahman and his colleagues[1] described a new synthesis of 6-methylenedihydrodeoxymorphine I. This compound was found to be somewhat more potent than morphine but had essentially the same pharmacological properties. In man the compound is said to have analgesic, sedative and respiratory depressant actions. Buckett[2] reported on the pharmacology of 14-cinnamoyloxycodeinone II the most potent series of esters of 14-hydroxycodeinone. The compound

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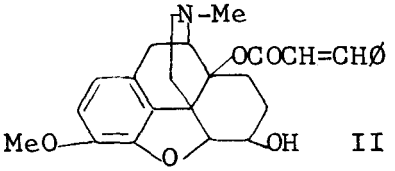
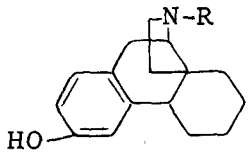


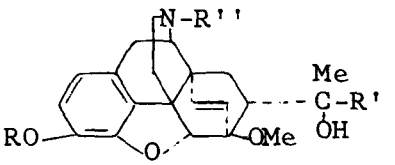

II

proved to be about 100 times more active than morphine as an analgesic with a better therapeutic ratio particularly by the oral and subcutaneous routes. In other pharmacological parameters II was qualitatively quite similar to morphine.

Gates and Klein[3] have prepared some metamorphinan derivatives (III) where the ethanimine bridge terminates at $C_{14}$ rather than $C_{13}$. The N-methyl derivative was tested as an analgesic and the N-cyclopropylmethyl compound as an analgesic




III

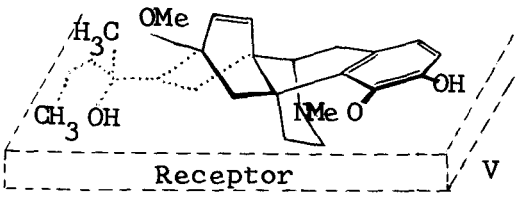



IV

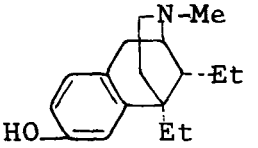
antagonist. Both compounds were essentially inactive, lending confirmation to the hypothesis of Braenden, Eddy and Halbach[4] that a phenyl group or group isosteric with phenyl connected directly to a quarternary carbon is a necessary structural feature of all strong analgesics.

B. Oripavine Analogs - Bentley and his colleagues[5,6] have continued their very interesting work in the 6,14-endoetheno-tetrahydrothebaine series of analgesics of the general structure IV. They have noted, in general, that if one keeps R and R'' constant, increasing R' from H through $CH_3$ and $C_2H_5$ to $C_3H_7$ leads to a progressive increase in analgesic activity. The N-substituents usually associated with narcotic antagonism (allyl, cyclopropylmethyl) give antagonists only when R' is H, $CH_3$ or $C_2H_5$. The most potent analgesics and antagonists are found in the phenolic series (R=H). Reduction of the 6,14-etheno bridge produced only slight changes in either analgesic or antagonistic activity. A reversal of the stereochemistry at C-19, although not extensively explored, revealed that varying the alkyl group R' now no longer effected analgesic activity. This indicated that the alcoholic group at C-7 might be a strong factor in attachment at the receptor surface. The marked decrease in analgesic activity noted with dehydration of this alcohol lends credence to this hypothesis.

On the basis of these and other studies Bentley and his colleagues[5,6] have proposed a hypothetical receptor site (V) which will accommodate these new molecules as well as the older analgesic structures.



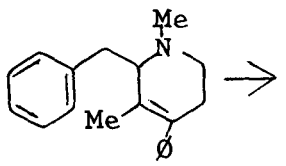

V

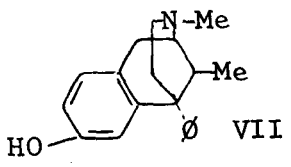



VI

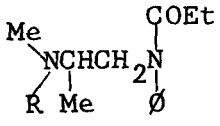
C. Benzomorphans - May and Eddy[7] have reported the resolution of cis-5,9-diethyl-2'-hydroxy-2-methyl-6,7-benzomorphan (VI). The (-)-isomer is as active as morphine and about twice as potent as the racemate in the mouse hot-plate test. The (+)-isomer also had analgesic activity, being 1/6 as potent as its antipode. Surprisingly, the (-)-isomer was found to precipitate withdrawal in addicted monkeys[8] and to have a mixture of analgesic and analgesic-antagonist properties in the mouse tail-flick test[9]. The (+)-isomer was found to have a definite physical dependence capacity in monkeys while the racemate was essentially inactive in this regard. This is the first finding of antagonistic activity in a N-methyl derivative and would suggest that the low physical dependence capacity of the racemate might be due to some form of antagonism between the two antipodes.

A similar case has also been reported by Clarke and his co-workers[10,11]. They have prepared a series of 5-phenyl substituted benzomorphans. In the case illustrated, there is good evidence to indicate that the ring closure goes trans. This is quite different from other similar ring closures in this series where the predominent product is the cis-isomer[12].




VII




VIII

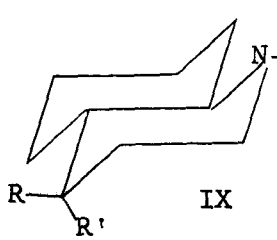
A: R=ØCH$_2$
B: R=ØCH$_2$CH$_2$
C: R=Me

In any case, the 5-phenyl compound VII appears much like a straightforward analgesic in the laboratory [10]. Despite this, the racemate has a low physical dependence capacity in monkeys and the (-)-isomer has the properties of a nalorphine-like antagonist[13]. Again, the (+)-isomer has a high physical dependence capacity. In a primary monkey addiction study (-)-VII produced only a very low grade physical dependence. This might be due to the low solubility (propylene glycol was necessary), absorption, and irritation associated with the drug. The anomalous behavior among the isomers in this and the 5,9-diethyl series may help explain the aberrant results consistently obtained with the benzormorphans in monkey dependence studies.

Keats[14] has evaluated (-)-VII in man for analgesic activity and found it to be approximately twice as potent as morphine. There was concomitant respiratory depression which was well antagonized by nalorphine. In addiction studies in man, Martin[15] has found that the compound produced miosis, was identified as "dope", and "liked" by post-addicts. It was also partially able to substitute for morphine in dependent subjects.

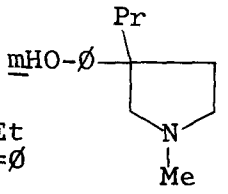
Clarke and his co-workers have also prepared a number of carbamates in the 5-phenyl series[16]. One of these, 2-carboxamide-2'-hydroxy-5-phenyl-6,7-benzomorphan, had weak analgesic activity in the hot-plate test and did not substitute for morphine in the addicted monkey[13]. This compound has been reported to have codeine-like analgesic activity in man[17].

D. Piperidine and Diphenylmethane Analogs - Sasaki and his colleagues[18] discussing the absolute configuration and conformation of some 1,2-diphenylethylamine derivatives reported that the analgesic activity of (-)-N,N-dimethyl-1,2-diphenylethylamine was consistent with its stereochemical resemblance to (-)-morphine. Nuclear magnetic resonance data for this compound indicated an eclipsed conformation to the extent of 22%. It was postulated that this might be expected to favor its approach to some reactive surface in the nervous system. Casey and Hassan[19] have prepared (RS)-, (R)-, and (S)-N-(2-dimethylaminopropyl) propioanilide (VIII A) and the benzylmethylamino analogs of methadone and isomethadone and reported pharmacological data for the various enantiomorphs of diampromid (VIII B) and the N,N-dimethyl derivative VIII C.




A: R=Ø, R'=OCOEt
B: R=OCOEt, R'=Ø

IX




X

The (S)-enantiomorphs of the anilides were more active than the (RS)- or (R)- forms and the arylalkyl compounds were considerably more active than the dialkyl compound. N-Benzylmethadone and N-benzylisomethadone had little activity. These results would indicate that the basic-anilide analgesics have a different mode of binding to the receptor than do the 3-amino-1,1-diphenylpropyl compounds.

E. Miscellaneous - Smissman and Steinman[20] reported the synthesis of the two isomeric forms of 1-methyl-4-phenyl-trans-decahydro-4-propionoxy quinoline (IX A and B). This rigid ring system varies only in the conformation of the aromatic ring and it was hoped that the biological activity of these compounds would resolve the controversy over whether an axial aromatic function was necessary for analgesic activity. The two compounds differed neither in their acute toxicity nor their analgesic $ED_{50}$. Thus, it would appear that no definite conformational requirement of the phenyl group is necessary for analgesic activity.

The pharmacologic properties of the prodilidine analog X was reported[21]. X was a considerably more active analgesic than prodilidine and its antinoci ceptive action was antagonized by nalorphine. The l-isomer was twice as potent as the d-isomer.

A considerable interest was shown last year in a steroid which was reported to have strong analgesic properties in both animals and man[22,23]. A recent paper[24] from the same laboratories has retracted these results.

Methotrimeprazine, a phenothiazine with strong analgesic activity, has received F.D.A. approval. The particularly careful study by Houde and his colleagues[25] sum up the experience with this drug. They find methotrimeprazine, unlike chlorpromazine, to be an effective analgesic. It is about one-half as potent as morphine and is associated with a high incidence of sedation and orthostatic hypotension. The drug, however, produced little nausea, respiratory depression, and has no morphine-like addiction potential. It represents a class of analgesics which differs qualitatively quite markedly from morphine.

F. Biochemical and Pharmacological Considerations - A number of papers have been published in an effort to shed more light on the mechanism of the strong analgesics at the molecular level. Casy and Wright[26] measured the ionization constants and partition coefficients of a number of 2-benzylbenzimidazoles. No good correlation was found between either of these parameters and analgesic activity. The intracellular distribution of dihydromorphine in the brain and liver of rats was studied by Praag and Simon[27]. They found most of the drug in the soluble fraction. A similar study by Mule[28] gave much the same results. Despite a careful analysis of most subcellular particles no specific morphine binding sites were found in either naive or tolerant animals nor did nalorphine effect tissue levels or intracellular distribution.

It has been reported[29] that morphine lowers the rate of incorporation of glucose into α-aminobutyrate and aspartate by rat brain vivo. In guinea pig cerebral cortex slices[30] high concentrations of morphine and nalorphine stimulated the incorporation of $P^{32}$ and $C^{14}$-labeled precursors into certain phospholipids. The incorporation into phosphatidylcholine, however, was inhibited. It is postulated that morphine and nalorphine may act by stimulating biosynthetic pathways leading to tissue depletion of D-1,2-diglyceride, an essential intermediate in the biosynthesis of glycerophosphatides.

Of great interest is the report by Kakunga et al.[31] who found that the intracisternal injection of $Ca^{++}$ suppressed the analgesic effects of morphine and other narcotics. EDTA and other decalcifying agents enhanced the action of the strong analgesics and counteracted the antagonistic effects of $Ca^{++}$. These results indicate that $Ca^{++}$ in the C.N.S. might be involved in the mechanisms by which opiates exert their analgesic effects.

The attempts to relate narcotic tolerance or action to protein or nucleic acid synthesis have continued[32,33,34]. To date, no satisfactory general theory has emerged from this work

It was reported[35] that morphine does not act as a sympatholytic on the isolated heart nor does it alter catecholamine levels or the response of the heart to sympathomimetic amines. On the other hand, in vivo experiments in the dog[36] reveal that morphine increased contractile force. This careful study revealed that the improved ventricular performance induced by morphine is indirect and probably the result of sympathoadrenal discharge.

The effect of various narcotic analgesics on thyroid function have been assessed and it was concluded that they did not exert a direct action on the thyroid but depressed thyroid function through an effect on the hypothalamic-pituitary axis[37]. This was further clarified in an elegant study by Lomax and George[38] whose results lead them to postulate that morphine acts on the thyroid by activating areas in the posterior hypothalamus which inhibit the release of TSH from the pituitary.

III. Analgesic Antagonists - Interest in this area, particularly from a clinical viewpoint, remains very high. Compounds of this class show great promise as nonaddicting analgesics and at least one of them appears to be quite effective in the treatment of addicts.

A. Pentazocine - The clinical reports on the use of this drug continue to look favorable. Pentazocine has been compared parenterally in controlled studies with phenazocine in post-operative pain[39], and morphine in cancer patients[40]. In both cases the drug gave good analgesia with a relatively low side effect incidence in the post-operative patients. In the cancer patients, who had a high level of prior narcotic use, a number of frank withdrawal episodes were observed. It is obvious that pentazocine will have to be used carefully in patients who have been receiving high doses of narcotics over a period of time. The effect of pentazocine on uterine contractility and fetal heart rate was also studied[41]. The drug produced an overall increase in uterine activity and did not retard labor. There were no significant changes in fetal heart rate. Pentazocine was stated to be an effective analgesic in labor with a rapid onset and short duration.

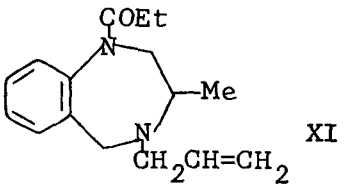
In a controlled post-surgical analgesic study[42] oral pentazocine was compared to codeine, aspirin, and a placebo. Pentazocine 50 mg was as effective as codeine 60 mg and more effective than 600 mg of aspirin. Pentazocine 35 mg was more effective than the placebo but less effective than the aspirin. It was found that the analgesic potency of the placebo was quite high and, as a second dose, was functionally dependent on the previous medication.

B. Cyclazocine - Cyclazocine is a potent narcotic antagonist which is also a potent analgesic in man associated with a high incidence of bizarre psychic effects. Analgesic doses of cyclazocine had little effect on blood pressure and heart rate in normal volunteers before or after tilting[43]. Marked CNS effects, however, were seen. On the basis of its behavior in addiction studies, cyclazocine was thought to be of use in the treatment of narcotic addicts[44]. The extensive trials initiated last year continue to be promising[45]. Indeed, continued research in this area was strongly recommended by an expert committee on dependence-producing drugs at the 21st session of the United Nations Commission on Narcotic Drugs.

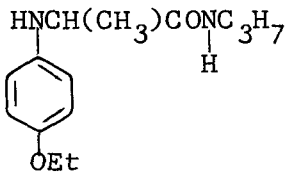
C. Naloxone - Naloxone, N-allylnoroxymorphone, has engendered a considerable amount of interest. Pharmacological studies would indicate that this compound represents the most nearly pure antagonist of the compounds yet tested. It is practically devoid of analgesic activity in animals[46,47]. The compound only rarely produces psychotomimetic effects and unlike nalorphine and cyclazocine shows few withdrawal signs after abrupt cessation of chronic medication[15]. The ability of naloxone to relieve pain in man is still in doubt[14].

D. Miscellaneous - The problem of devising a laboratory test capable of predicting the clinical analgesic activity of the narcotic antagonists has yet to be solved. The inhibition of writhing[47] does not appear to be sufficient nor does the escape response elicited by tooth pulp stimulation[48]. The narcotic-antagonist analgesics do depress the coaxially stimulated guinea pig ileum and naloxone is ineffective in this regard[9,49]. It has been reported that cholinergic mechanisms may be involved in the action of these compounds[9,50]. Thus, the previously inactive narcotic-antagonist analgesics are capable of blocking the tail-flick reflex in the presence of physostigmine. Cholinesterase levels in the brain also appear to be altered by these agents.

Analgesic antagonist activity has been reported[51] in a series of 4-substituted 1-acyl-2,3,4,5-tetrahydro-1H-1, 4-benzodiazepines XI. Although these compounds are only weak antagonists they represent the first narcotic antagonists derived from a series devoid of analgesic activity.




XI




XII

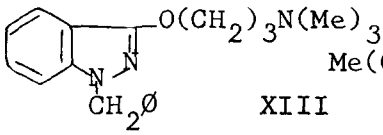
IV. Weak Analgesics - Since there is a considerable overlap between weak analgesics and Non-Steroidal Anti-Inflammatory Agents, Chapter 21 should also be consulted.

A. Salicylates - There was a continued interest in ascertaining the cause of gastric bleeding after the ingestion of aspirin and other salicylates. Gastric erosions appear to result when the rate of loss of surface cells exceeds the replacement rate[52]. Sodium salicylate induces a shearing of the villus epithelium from the underlying mucosa[53] and interferes with mucoprotein synthesis in the gastrointestinal tract[54]. These changes may be caused by a blood borne factor[55].

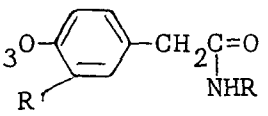
An interesting laboratory test for mild analgesics involves measuring the influence of drugs on recovery from pain-induced motor impairment in dogs[56]. A pharmacokinetic study of salicylate elimination[57] helps relate the rat to man.

B. Aniline Derivatives - A structure-activity study of a new series of alkoxyaniline derivatives has been reported along with a detailed workup of one of them[58]. This compound (XII) appears to have good analgesic and anti-inflammatory activity, associated with some CNS depressant effects. An antidiuretic effect was described for acetaminophen[59] and it was suggested that this drug might be a useful substitute for vasopressin in certain patients with diabetes insipidus. Evidence was also presented[60] to show that acetophenetidin has antipyretic activity which is not dependent on metabolism to N-acetyl-p-aminophenol.

C. Pyrazolone Derivatives - Some pyrazolone derivatives were prepared which had analgesic activity[61]. Despite interesting structure-activity relationships, none of these compounds were markedly more effective than pyramidon. An interesting variation is found in a series of indazoles[62]. Those with a dimethylaminopropoxy side chain were the most active. One of these, benzydamine (XIII) has been extensively studied




XIII



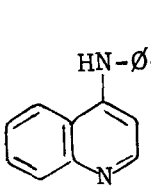

XIV

A: R=OH, R'=Me
B: R=R'=H

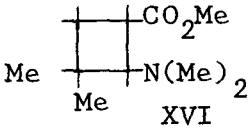
pharmacologically[63] and is currently under clinical trial.

D. Miscellaneous - Buu-Hoi and his colleagues[64] have prepared and tested a series of arylacethydroxamic acids (XIV). Compound XIV-A had both anti-inflammatory and analgesic activity while the acetamide XIV-B was devoid of anti-inflammatory activity yet retained the analgesic properties. These compounds are also being assessed in man.

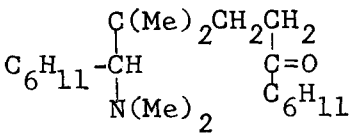
A quinoline derivative, Glaphenin, (XV) is reported to be 5-10 times more active than aspirin as an analgesic in animals[65]. The compound appeared to have little effect on the CNS, autonomic, and respiratory systems. Some δ-amino ketones were also reported to have analgesic activity[66]. The cyclobutane derivative (XVI) represents the first instance of analgesic activity for a structure containing a non-aromatic cyclobutane ring. The analgesic effects of XVI are not antagonized by nalorphine. When a Grignard reaction was attempted with XVI, the ring cleaved, resulting in two types of δ-amino ketones. Several compounds of this type had analgesic activity




XV




XVI




XVII

and one of them, (XVII) had analgesic activity which was antagonized by nalorphine. Compound XVI has been studied in the clinic and seems to exhibit analgesic and side effects similar to aspirin.

One of the great difficulties encountered in the study of mild analgesics remains in their clinical evaluation. An experimental pain study utilizing electrical and thermal stimuli was unable to distinguish between aspirin and a placebo[67]. That pathological pain studies are also not wholly the answer was revealed in a study by Kantor et al[42].

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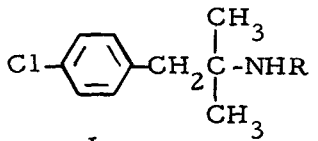
## Chapter 5. Anorexigenic Agents

George I. Poos, McNeil Laboratories, Inc., Fort Washington, Pa.

No breakthroughs were evident in the field of anorexigenic agents during 1966. Research continued to be concentrated on drugs related in structure to amphetamine.

Gylys[1] compared the effect of chlorphentermine (Ia) and amphetamine on food motivated operant behavior in rats. At equi-anorectic doses, a greater depressant effect on several food motivated schedules was found for chlorphentermine than for amphetamine. Schmitt[2] studied the EEG records of rabbits treated with chlorphentermine. At doses of 2.5-10 mg./kg., which were sedative, high voltage slow waves were produced. There was blocking or shortening of the arousal reaction produced by stimulation of the reticular activating system while the recruiting reaction from stimulation of the antero-medial thalamic nucleus was facilitated.



I

a R = H

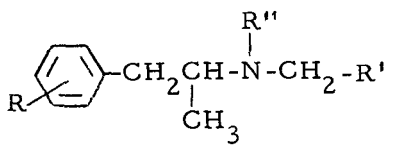
b R = $CO_2C_2H_5$

An effort to obtain objective measures of the CNS effects of chlorphentermine in humans was unsuccessful.[3] Using the "alpha blocking response" (effect of flashes of light on the EEG alpha rhythm) in normal subjects, chlorphentermine showed no effect in 65 mg. doses. However, d-amphetamine (15 mg.) and pentobarbital (100 mg.) were also undistinguishable from placebo in this study. Another potentially useful objective method to determine CNS stimulation or depression of anorectics clinically is that of measuring the "critical flicker fusion frequency". In this test, depressants decrease and stimulants increase the discernable frequency of a flickering light (normally ca 32 cycles/sec.).[4, 5, 6]

From a survey of the clinical experience with chlorphentermine to date, it appears that at effective anorectic doses there is a low but about equal incidence of both depressant and stimulant CNS side effects.[7, 8]

The N-ethylcarbamate of chlorphentermine (Ib, cloferex) is reported to be a less toxic, longer acting compound than the parent base.[9, 10]

A series of benzphetamine (IIa) analogs IIb in which R was H, 4-Cl and 3-$CF_3$, R' was phenyl, halophenyl, furyl, thienyl, etc., and R" was H



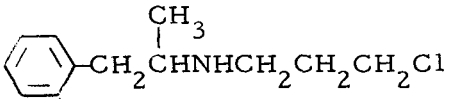
II

| | R | R' | R'' |
|---|---|---|---|
| a. | H | phenyl | H |
| b. | H, Cl, $CF_3$ | phenyl, subst. | H |
| | | phenyl, heteroaryl | $CH_3$ |

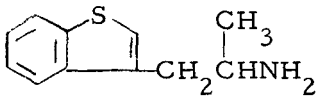
and methyl were reported as anorexigens[11] in rat and dog tests. Activities between phenmetrazine and d-amphetamine were reported. Several striking species differences were found.

N-allyl and N-propargyl derivatives of various methamphetamines are claimed in a Swiss patent to have appreciable anorexigenic activity in rats.[12] Several methylated derivatives of phenmetrazine and ephedrine were shown to have no anorectic advantage over the parent compounds.[13]

Clinical trials of RO 4-5282 (N-3-chloropropylamphetamine III) at daily doses of 47 to 140 mg. revealed effects similar to those obtained with phenmetrazine.[14, 15]



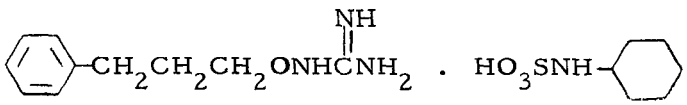
III



IV

Rapidly developing anorectic tolerance in rats has been reported for p-ethyl- and p-trifluoromethylamphetamine as well as 3-(2-aminopropyl)benzo[b]thiophene (IV, SKF 6678).[16] Cross tolerance with d-amphetamine was not found. The three compounds studied have reduced CNS stimulant properties and it was suggested that more rapidly developing tolerance may be a general property of the less stimulant anorectics.

A new aralkoxyguanidine V (U-16, 178 F) has been reported to be about 1/5th as potent as d-amphetamine in causing weight loss and about

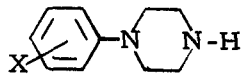


V

1/12th as active in inhibiting food intake in mice.[17] No stimulant activity was found in mice for V up to 40 mg./kg., I.P. It blocked maximal electro-shock in mice ($ED_{50}$ 89 mg./kg.) and caused a transient depressor effect in dogs at 16 mg./kg., I.V.

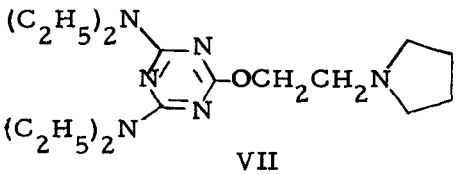
1-Phenylpiperazine and its m- and p-chloro and methyl derivatives (VI) were claimed to have about one-half the anorexigenic activity of am-

phetamine with much less acute toxicity and no CNS stimulation.[18]



VI

X = m and p Cl and $CH_3$



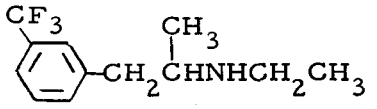
VII

Various diamino-S-triazines with amino alkoxy side chains were claimed in a Dutch patent to be very potent anorexigenic agents with low toxicity and a lack of CNS stimulation. An $ED_{50}$ of 0.3 mg./kg. and $LD_{50}$ of 180 mg./kg. S.C. in mice was given for the most potent compound, 2,4-bis-diethylamino-6-(2-pyrrolidylethoxy)-1,3,5-triazine (VII).[19]

The effects of anorexigenic agents on lipid metabolism continued to receive attention. Fassina[20] suggests that by increasing plasma free fatty acids, anorexigenic drugs reduce fat stores and by elevating lipid metabolism, decrease appetite in accord with the lipostatic theory of appetite regulation. On the other hand, when the free fatty acids in plasma were held at a low level by the administration of 3,5-dimethylisoxazole, the anorectic effect of methamphetamine in rats was not reduced.[21]

Further reports on the effect of anorexigenic agents on other metabolic factors such as blood glucose,[22] liver glycogen,[22] oxygen consumption[23] and plasma insulin[24] appeared. Whether these effects are secondary or are directly involved in anorexigenic action has not been resolved.

An investigation of urinary excretion in rats showed that 67% of chlorphentermine (Ia) was excreted unchanged after a 20 mg./kg. dose.[25] Cloferex (Ib) administration in rats led to the partial (39%) excretion of chlorphentermine. Phentermine and fenfluramine (VIII)[26] were found in the urine to an extent of only 4% and 6%, respectively,[25] after administration to rats.



VIII

Interesting central anti-amphetamine effects are described for tyrosine hydroxylase inhibitors such as α-methyltyrosine.[27] Since norepinephrine depletors do not antagonize amphetamine and tyrosine hydroxylase inhibitors have little direct sedative effect, it is suggested that small but critical levels of norepinephrine at receptors are necessary for amphetamine to exert both its stimulant and anorexigenic effects. Whether this applies to other anorectic drugs remains to be determined.

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Section II - Pharmacodynamic Agents
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Rensselaer, New York

## Chapter 6. Antihypertensive Agents

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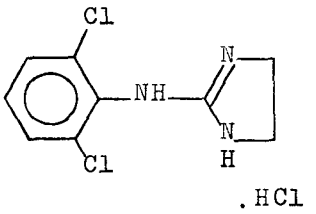
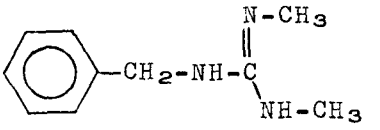
Catapresan® - The most significant development in the field of antihypertensive agents during 1966 was the publication of extensive and detailed investigations of a new antihypertensive agent, 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride (I) (Catapresan®).[1-13] This compound was designed and tested initially as a vasoconstrictor. Preliminary clinical testing by intranasal administration produced marked prolonged sedation, a lowering of blood pressure, bradycardia and dryness of the mouth. These observations led to extensive investigations of the substance in experimental animals and in man which established that the marked antihypertensive effect was its most important property. Two hundred and fifty imidazoline derivatives were synthesized and tested pharmacologically, but none was more effective than Catapresan®. Slight structural changes considerably diminished or abolished the blood pressure lowering effect. Clinical testing of twenty analogs was carried out, however, all of these were found to be less active than the parent compound.[5]




.HCl

I




.1/2 $H_2SO_4$

II

Catapresan® shows a structural resemblance to guanethidine type compounds, particularly bethanidine (II), and to imidazolines both of the vasoconstrictor class such as

xylometazoline, and also to phentolamine, noted for its blood pressure lowering effect.[5]

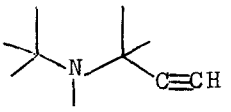
Pharmacological tests[1] showed that upon intravenous administration to dogs in a dosage range of 10-30 γ/kg a sedative effect was produced along with a blood pressure decrease of long duration accompanied by bradycardia, inhibition of blood pressure raising cardiovascular reflexes and decrease of cardiac output. Contraction of the nictitating membrane in cats, induced by preganglionic electrical stimulation, was not inhibited. No adverse effects on the myocardium were seen in experiments with the heart-lung preparation. The compound did not cause a decrease in the noradrenaline content of the rat heart. Some sympathomimetic effects were observed in the lower dosage range and inhibition of ulcer formation and acid secretion in the rat stomach were observed mainly with doses higher than 30-100 γ/kg.

Toxicological studies, both subacute and chronic in rats and dogs, and teratologic experiments in rats, mice and rabbits were conducted with Catapresan® and the results described as favorable even with high dose levels and after long administration[4]. $C^{14}$ studies[3] showed that the compound is readily absorbed by the rat after oral administration. Two-thirds of the dose was excreted in the urine and one-third in the feces. Elimination was apparently complete during the first forty-eight hours with 60-70% of the substance excreted unchanged.

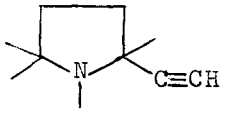
Clinical studies on Catapresan® have been reported[6,10,11,13] which indicate that a reliable and substantial lowering of blood pressure can be obtained upon oral administration in divided doses of 225-1350 γ of the drug to hypertensive patients. The effects can be seen in cases of primary and secondary, benign or malignant hypertension[13]. The degree of pressure reduction attainable was about the same as that from α-methyldopa[13]. Side effects encountered were dryness of the mouth and sedation, particularly at higher doses.[6,10,11,13] A slight, dose related decrease of the pulse rate was noted.[10] There was little effect on orthostatic blood pressure[6,11,13] and few digestive disturbances.[6] Reports on the effect of the drug on renal function were not entirely consistent. Both unchanged[13] and reduced[11] p-aminohippuric acid and inulin clearances were reported. No notable change in urine volume and excretion of electrolytes was seen[6] but in renal and essential hypertension, a statistically significant rise of the serum concentration of sodium and chloride ions occurred. The antihypertensive effect of Catapresan® was potentiated by the addition of drugs which promoted renal salt excretion.[13]

Some interesting comparisons have been drawn between the characteristic actions of Catapresan® and reserpine, α-methyldopa and guanethidine.[5] Similarities and distinctions were noted in each case. The conclusion was reached that the mechanism of action of Catapresan® has not, as yet, been clarified and the drug has characteristics such that it cannot be adequately classified in any of the known drug groups now used in antihypertensive therapy.[5]

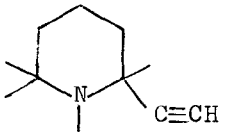
Ganglionic Blocking Agents - A number of cyclic hindered amines substituted on the α-carbon with ethynyl, vinyl and alkyl groups have been synthesized and their antihypertensive properties compared with a corresponding series of open chain compounds. It was found that the open chain compounds were




III




IV



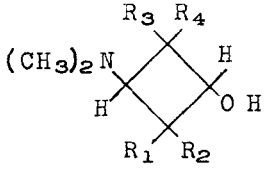

V

generally more potent in their hypotensive effect. For example, 2-ethynyl-1,2,5,5-tetramethylpyrrolidine (IV) and 2-ethynyl-1,2,6,6-tetramethylpiperidine (V) produced a mean average pressure drop in hypertensive rats of 6% at 20 mg/kg and 5% at 5 mg/kg, respectively, compared to a pressure drop of 11% at 5 mg/kg produced by the open chain analog 3-(N-t-butylmethylamino)-3-methyl-1-butyne (III). The results appear to be consistent with the hypothesis that the principal factor controlling activity is the degree of shielding of the basic nitrogen atom provided by alkyl groups substituted on the two adjacent carbon atoms[14].

The ganglionic blocking action of several methylpiperidines as their hydrochlorides or quaternary methosalts has been studied. The most potent compound was the secondary 2,2,6,6-tetramethylpiperidine. The ganglionic blockade produced by secondary and tertiary amines was found to be of long duration. The quaternary compounds were more active in equimolar doses but their effects were of shorter duration. It was demonstrated that the presence of at least three methyl groups on the α-carbon atoms of the piperidine ring is not absolutely necessary to confer ganglionic blocking properties on the molecule, although it has been confirmed that increasing the number of methyl substituents of the 2-

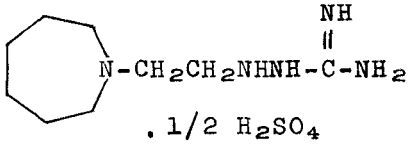
and 6- positions augments this effect. The ganglionic blockade produced was shown to be of the nondepolarizing type.[15]

A series of cyclobutanolamines (VI, where $R_1R_2R_3$ and $R_4$= H or alkyl) has been found to possess ganglionic blocking and hypotensive activity and the importance of the effect of the alkyl groups surrounding the basic nitrogen on activity has been demonstrated. More detailed studies on one member of the series, trans 2,2,4,4-tetramethyl-3-dimethylaminocyclobutanol (VI, $R_1=R_2=R_3=R_4=CH_3$) (RP 904) were described. This compound, which underwent rapid oral absorption, was shown to block preganglionic stimulation and to induce peripheral vasodilatation.[16]



VI

Guanethidine Analogs - β-Heptamethyleneiminoethylaminoguanidine sulfate, EX4891A, (VII) has been synthesized and tested on the basis that interposition of the amino group before the quanidine moiety in the straight chain position of the molecule might reduce or eliminate the acute sympathomimetic action of guanethidine. This possibility may be realized since the compound depleted catecholamines from the rat ventricle but did not exhibit a sympathomimetic effect due to acute release of catecholamines in active forms thus differing in the latter respect from guanethidine. Another distinction was that the tyramine-like effect of guanethidine, seen after parenteral administration, was not obtained with VII. Otherwise, the pharmacologic effects of this analog appear to be identical with those of guanethidine in producing adrenergic neurone blockade, having no apparent effect on out-flow from central sympathetic structures and showing transient direct vasodilatory effects.[17]

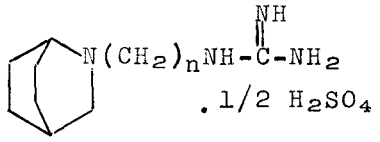


VII

Cis-cinnamyl guanidine sulfate, Su 13686, was found to be an orally active hypotensive agent in both anesthetized and unanesthetized normotensive dogs and in unanesthetized renal hypertensive dogs. Onset of action was rapid. The compound also reduced the blood pressure of anesthetized cats and rabbits. The pressor effects of epinephrine and norepinephrine were enhanced, whereas those from amphetamine were inhibited. Daily oral administration of 2 mg/kg of the drug to unanesthetized renal hypertensive dogs for twelve days reduced

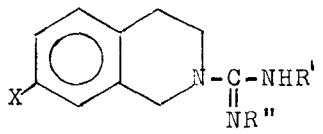
mean arterial pressure by 21 mm Hg.[18]

As part of a program to explore the effect of substituting the isoquinuclidine moiety for other groups in various drugs, N-[2-(guanidino)ethyl]- and N-[3-(guanidino)propyl] isoquinuclidine sulfates (VIII, n=2,3) were synthesized as analogs of guanethidine. Both compounds, when evaluated by intravenous administration to the anesthetized dog, were found to be less active than guanethidine in lowering blood pressure and to cause severe side effects.[19]



VIII

The pharmacological properties of N'N"-dimethyl-1,2,3,4-tetrahydroisoquinoline 2-carboxamidine hydrochloride, P4746, (IX, R'=R"=$CH_3$,X=H) have been described. This compound, which is the N'N" dimethyl derivative of debrisoquin (IX, R'=R"=X=H), was found to lower the blood pressure of conscious hypertensive dogs on chronic administration. Other observations indicated that this action is probably due to a reduction in sympathetic tone as a consequence of post-ganglionic sympathetic blockade. In acute experiments the compound elicited a hypertensive response, similar to guanethidine. It was less effective than guanisoquin (IX, $R^1$=R"=H, X=Br) in lowering myocardial norephinephrine levels.[20]



IX

The cardiac and renal hemodynamic effects of debrisoquin sulfate have been studied in hypertensive patients and found to be similar to those observed with guanethidine and the ganglionic blocking agents. Lowered blood pressure associated with reduced cardiac output and renal blood flow are apparently characteristic of the hemodynamic pattern of inhibition of the peripheral sympathetic nervous system.[21]

A new clinical study of guanoclor, {[2-(2,6-dichlorophenoxy) ethyl] amino} guanidine sulfate, in 35 hypertensive subjects has been described.[22]

Part I of a review on guanethidine covering the discovery, development, chemical structure, physical properties and pharmacological actions of this drug has appeared.[23]

Pargyline - The effect of pargyline on blood pressure in

spinal and decerebrate cats has been studied. It was found that the acutely administered drug produced a slowly developing hypertensive effect of long duration in spinal cats but under similar conditions in decerebrate animals, there was no effect on blood pressure. These experiments were held to indicate that the presence of the medullary vasomotor center is necessary to prevent the peripheral hypertensive action of pargyline.[23] A report appeared dealing with the pharmacodynamics of pargyline in the rat and cat.[24] A clinical study concerning the effect of pargyline on blood pressure and mood of 83 hypertensive patients with moderate to severe essential hypertension was described.[25]

Mebutamate - The mechanism of the hypotensive action of mebutamate was investigated in cross circulation experiments in anesthetized dogs. The results suggested that the initial and transient hypotension may be largely due to the direct vasodilator action and that the sustained effect may be accounted for by an inhibition of the sympathetic vasomotor tone at the spinal and ganglionic levels.[26]

Amino Acid Enzyme Inhibitors - A comparative clinical study of the antihypertensive effects elicited by intravenous injection of α-methyldopa and α-methyl-m-tyrosine has shown, contrary to previous reports, that the latter agent has a longer duration of action in essential hypertonia and also a more pronounced orthostatic effect.[28]

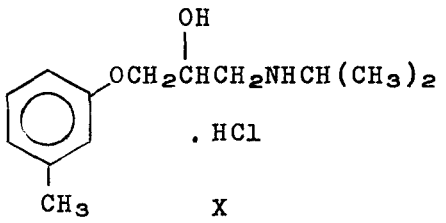
Findings of autoimmune haemolytic anemia associated with α-methyldopa therapy have been described[29,30] and a case of hypertensive response to α-methyldopa has been reported.[31]

Antihypertensive Diuretic Drugs - A review of the treatment of hypertension with antihypertensive diuretic drugs has appeared.[32] In a clinical study in 21 patients on triamterene (2,4,7-triamino-6-phenylpteridine), the drug had an inconsistent antihypertensive effect on the systolic blood pressure which was minimal in most patients, but in combination with hydrochlorothiazide appeared to be useful in providing a slight additional systolic blood pressure reduction and an increase in serum potassium levels.[33]

Diazoxide - It has been reported that 14 of 16 patients with severe chronic hypertension, who had become unresponsive to α-methyldopa, guanethidine and hydralazine, responded to a 20 day intravenous regimen of 300 mg doses of diazoxide with a substantial mean arterial pressure drop accompanied in some cases by clearance of retinopathy, decrease of cardiac size and lessening of congestive heart failure. Subsequently the blood pressure could be controlled by administration of chlorthalidone plus reserpine for a period of 30 months. Thus it

would seem that acute repeated reductions of arterial pressure during the 20 day period of diazoxide administration modified the severity of the vascular disease possibly by readjusting the so-called pressure barostat.[34,35]

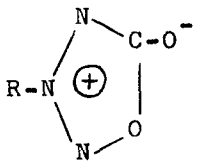
Beta Adrenergic Receptor Blocking Agents - The proceedings of a symposium on this subject have been published in which the use of propanolol (1-isopropylamino-3-(1-napthoxy)-2-propanol hydrochloride) in hypertension is discussed.[36] The pharmacological properties of a new adrenergic β-receptor antagonist, 1-isopropylamino-3-(3-tolyloxy)-2-propanol hydrochloride (ICI 45,763) (X) have been described.[37]




X

Peptides - Some bradykinin analogs have been synthesized and biologically evaluated. The 8-m-trifluoromethylphenylalanine analog was found to be about 1.5 times as active as bradykinin in lowering guinea pig blood pressure but only one-half as active in lung bronchoconstriction, thus supporting the idea that different receptor sites are involved for the hypotensive and bronchoconstrictive effects.[38] The proceedings of a symposium on hypotensive peptides have been published.[39]

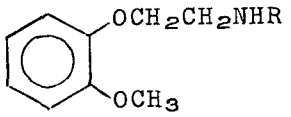
Other Antihypertensive Agents - A series of mesoionic compounds known as sydnone-oxatriazoles (XI) has been synthesized and shown to produce a hypotensive effect when administered intravenously to anesthetized dogs at a dose of 5 mg/kg. The blood pressure reduction ranged from about 6% (R=1,3-dimethylbutyl) to 30% (R=cyclohexyl) and the duration of action from one to two hours. Some of the hypotensive effects of these compounds may be due to competition with norepinephrine for adrenergic receptors.[40]




XI

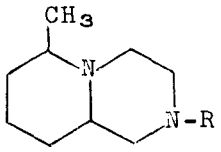
Some N-substituted phenoxyethylamines (XII) have been prepared and their hypotensive effects evaluated in anesthetized normotensive cats and neurogenically hypertensive dogs. A study of the structure-activity relationships revealed that the 2-(2-methoxyphenoxy) ethylamino moiety is necessary for maximum effect. Three members of the series; XII, R = $CH_2CHOHCH_2OCH_2CH{=}CH_2$, $(CH_2)_4C_6H_4OCH_3$ p and $(CH_2)_3OC_6H_3$-2,5

$(OCH_3)_2$, were subjected to clinical trials in which antihypertensive activity was observed.[41]



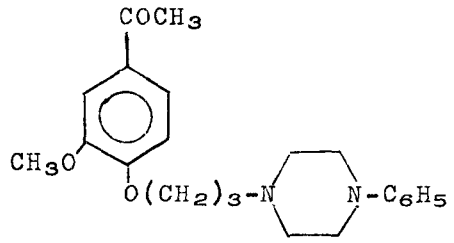
XII

A number of substituted 1,4-diazabicyclo[4.4.0]decanes have been synthesized and shown to lower the blood pressure of 5,5-diallylbarbituric acid - anesthetized cats upon intravenous administration. It appears that the hypotensive activity is primarily attributable to the 1,4-diazabicyclo [4.4.0]decane system but may be quantitatively modified by the nature of the substituents. The most potent compounds in the series are XIII, R = $CH(C_6H_5)_2$ and $CH_2CH_2NH_2$. The mechanism of action remains to be determined but probably is not ganglionic blockade.[42]



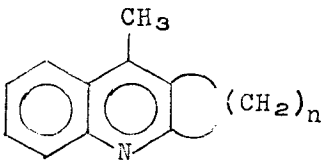
XIII

Experimental results relating to the hypotensive activity of 3'-methoxy-4'-[4-(4-phenyl-1-piperazinyl)butoxy] acetophenone (XIV) as the monohydrochloride (Su-14542) or dihydrochloride (Su-13396) in normotensive and renal hypertensive dogs have been reported. These were interpreted as indicating that Su-14542 does not act by ganglionic blockade but does possess β-adrenergic stimulating activity.[43] Other investigators presented data from which they concluded that Su-14542 is a potent α-receptor blocking agent but not a stimulant of the β-receptors of cat, rabbit or dog hearts. It was suggested that the heart stimulation previously observed following administration of Su-14542 is mediated reflexly as compensation for the fall in arterial blood pressure resulting from the intense α-receptor blockade produced by this compound.[44] The effects of Su-14542 on cardiac output and coronary blood flow[45] and on femoral renal blood flow[46] have been described.



XIV

Some condensed alicyclic lepidines (XV, n=3,4,5) have been the subject of a pharmacological study in comparison



XV

with lepidine (4-methylquinoline) itself and shown to exhibit a hypotensive response in rats and cats.[47]

A new clinical study of the antihypertensive action of progesterone in 17 hypertensive patients has been reported. A significant decrease of both systolic and diastolic blood pressures upon daily administration of 100 to 300 mg of progesterone intramuscularly was observed. The lowering of blood pressure was related to a reduction of peripheral resistance. The authors do not propose progesterone as a therapeutic agent for arterial hypertension but believe that its action is of interest in considering the pathological physiology of primary hypertension.[48]

General - A theory of hypertension, in which a set of existing ideas have been integrated in a new way, has been presented where it is suggested that adaptation of the baroreceptors to the high arterial pressure might be the primary factor in the disease. The clinical features of benign and malignant hypertension and the change from one to the other could be accounted for on this basis. Therapeutic implications of the hypothesis are considered,[49] and in this regard it is interesting to consider studies previously discussed.[34,35]

The role of renin in renal hypertension has been discussed and a hypothesis put forward containing the novel proposal that the blood pressure lowering capacity of the kidney is directly proportional to its renin content.[50]

A twenty year clinical follow-up study on essential hypertension was reported.[51]

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# Chapter 7. Diuretic Agents

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A large volume of research pertaining to diuretics was published in 1966. Many publications were concerned with chemical and patent reports which were largely extensions of series with known diuretic activity. Others involved biological and clinical studies regarding the mode of action and extra-renal effects of established diuretics. Although novel structural types with diuretic properties have been reported and interesting fundamental research on electrolyte transport and mechanism of drug action has been described, no obvious breakthrough has occurred during this year.

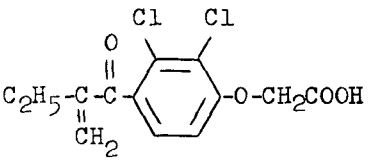
General. Noteworthy reports[1] and reviews pertaining to the pharmacological,[2] endocrinological[3,4] and clinical[5] aspects of diuretics have appeared in the recent literature. The use of diuretics in the treatment of hypertension has been reviewed with especial emphasis on the hypotensive action of the aldosterone antagonist, spironolactone.[6] Fundamental studies on the mechanism of transport of electrolyte across the tubular epithelium have indicated that phospholipids may play a critical role. Phospholipase C and pancreatic lipase markedly reduced the rate of reabsorption of saline droplets infused into rat proximal tubules. Likewise, phospholipase C reduced the ability of extractable lipids to bind sodium and potassium ions in rat kidney homogenates; whereas, phospholipase D and ribonuclease appear to enhance cation binding.[7]

Organomercurials. Although the synthesis of new organomercurial diuretics is apparently at a standstill, interest continues in the mode of action of agents of this class. A variety of experimental techniques have been used in attempts to define more clearly the site of action of the mercurial diuretics in the kidney tubule. Recent stop flow studies[8] with meralluride have shown that the mercurial can inhibit sodium reabsorption in the distal tubule. The magnitude of this effect bears a direct relationship to plasma sodium concentration. Since furosemide produces an added response during maximal mercurial natriuresis in normal but not in acidotic dogs, it was suggested[9] that, in the acidotic state, the action of mercurials is extended to the ascending limb of Henle's loop, considered to be the major site of action of furosemide.

Studies on the distribution of $^{203}$Hg-chlormerodrin have already demonstrated that the cells of the proximal convoluted tubule contain the maximal concentration of mercury during diuresis in the dog. It has now been shown[10] that a similar distribution of mercury results when diuresis is prevented by metabolic alkalosis or administration of p-chloromercuribenzoic acid. Therefore, the tubular binding of chlormerodrin need not be determined solely by factors involved in the production of diuresis, but

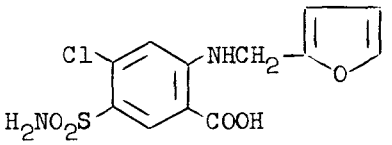
may be more closely related to tubular secretion of the drug. The distribution patterns of $^{203}Hg$-mersalyl have also been described.[11]

Acylphenoxyacetic Acids. Ethacrynic acid (I), the powerful saluretic effect of which has been documented, was made available commercially in the United States in January, 1967. Additional studies on the sites of action of I showed that it probably inhibits reabsorption of sodium and chloride in the proximal tubule as well as in the ascending limb of Henle's loop.[12,13] It was demonstrated in the perfused dog kidney that I reduces renal vascular resistance in contrast to the thiazides and mercurials which increase resistance.[14] Ethacrynic acid markedly increases bromide excretion and has been recommended for the therapy of bromide intoxication.[15]

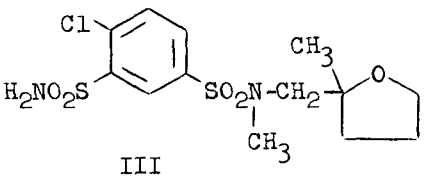


I

Aromatic Sulfonamides. A considerable volume of biological and clinical literature continues to appear regarding furosemide (II) which was made available to the United States' market in 1966. In addition to



II



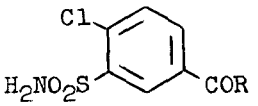
III

the publications concerning its use in a variety of clinical situations, some fundamental facts concerning its pharmacological properties have been elucidated. Stop-flow studies in man have confirmed the animal data which indicated that the site of action of the drug was the loop of Henle and early distal tubule.[16] Furosemide was reported to decrease renal vascular resistance in the kidney of anaesthesized dogs receiving a constant flow of femoral artery blood. The drug also increased renal blood flow in animals with intact kidneys. Hydrochlorothiazide and chlorothiazide showed the opposite effects while ethacrynic acid was similar to furosemide. These observations offer a hemodynamic basis for the unique effects of furosemide and ethacrynic acid since, in addition to their renal tubular effects, they appear to increase renal blood flow by vasodilation.[14] From toad bladder studies, it was concluded that furosemide has no direct effect on the sodium pump, but may act as an antagonist of cyclic 3',5'-AMP.[17] Although the effect is rather small, furosemide does appear to reduce blood pressure in man.[18-20]

2-Chloro-5-[N-methyl-N-(2-methyltetrahydrofurfuryl)sulfamoyl]benzenesulfonamide (mefruside, FBA 1500, Bay 1500) (III) was reported to be a potent diuretic in mice, rats, dogs and man. The lowest effective oral

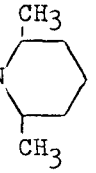
dose in hydrated rats was 0.05 mg./kg. and there was a linear increase in urinary sodium chloride and water as the dose was increased to 320 mg./kg. Maximum saluresis considerably above that of the thiazides was described. Mefruside, in contrast to hydrochlorothiazide, was effective in hepatectomized rats. It is metabolized to a lactone derivative which also has diuretic properties.[21,22]

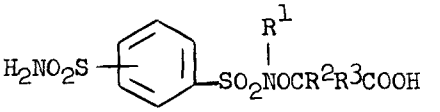
Earlier work[23] on 3-sulfamoyl-4-chlorobenzamide, IV, (sulclamide) has been extended to include the corresponding benzoic acid, V. Both compounds were active in rats at 2 mg./kg. orally, but at 50 mg./kg. V is more potent. They were effective in dogs in the range of 5 to 30 mg./kg. i.v. and 50 to 200 mg./kg. p.o.[24]




IV, R = $NH_2$

V, R = OH

VI, R = 


VII

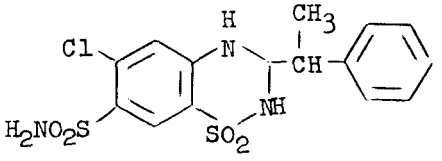
A number of hydrazides of 3-sulfamoyl-4-chlorobenzoic acid were studied for possible interactions with catecholamine-dependent functions. Only the cis-N-(2,6-dimethyl-1-piperidyl)-3-sulfamoyl-4-chlorobenzamide (VI, Clopamide, Brinaldix) was active. It inhibited reserpine-induced hypothermia in mice and increased the sensitivity of rabbit aorta strips to noradrenaline.[25]

In a series of compounds of type VII, the most active in rats were those in which the sulfamoyl group was in the para position and $R^1$, $R^2$ and $R^3$ respectively were (1) H, H, H, (2) $CH_3$, H, H and (3) H, $CH_3$, $CH_3$.[26]

Benzothiadiazines. A large volume of literature continues to be generated regarding the thiazides, some of which is only of archival value. Thiazide-induced hyperglycemia has been correlated with potassium loss,[27,28] although more evidence will be required to validate this finding. Indeed, abnormal glucose tolerance curves resulting from chlorothiazide therapy are reported to be normalized by potassium chloride or triamterene administration.[29] However, the hyperglycemia due to hydrochlorothiazide in nephrectomized-alloxanized rats was related to increased glycogenolysis caused by inhibition of cyclic 3',5'-AMP phosphodiesterase; the resultant accumulation of cyclic 3',5'-AMP increased the hyperglycemic effect of epinephrine.[30] Other causes of thiazide-induced hyperglycemia have been suggested including reduced insulin secretion[31] and impaired

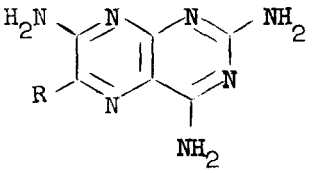
glucose uptake by peripheral tissues.[32]

Since reports of new thiazides which have reached the clinic are rare in recent years, a new addition is worthy of mention. This compound, the 3-α-methylbenzyl derivative of hydrochlorothiazide (VIII), is referred to in the literature as Diu 60 (Dehydrosanol, Pertensosonol). Preliminary reports on its chemistry have appeared previously,[33,34] but detailed reports of its toxicological[35] and diuretic properties in animals are now available which indicate that it compares favorably with other thiazides in animals and in man.[36]

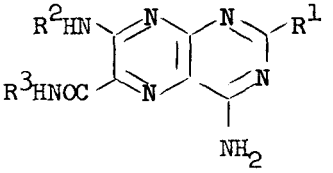


VIII

Pteridines. The diuretic properties of triamterene, 2,4,7-triamino-6-phenylpteridine (IX, R = $C_6H_5$), are well known. A number of related compounds (IX) in which the 6-phenyl group was replaced by a variety of cycloalkenyl groups and by furyl were found to have diuretic activity in rats about equal to that of triamterene.[37] One compound of this group,



IX



X

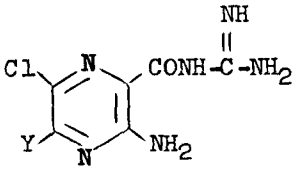
2,4,7-triamino-6-(2-furyl)pteridine (furterene, IX, R = 2-furyl), in the form of its formic acid salt was selected for detailed study because of its low toxicity. Its action in promoting sodium and chloride excretion while suppressing potassium excretion resembles that of triamterene. The persistence of activity in the adrenalectomized rat shows that furterene, like triamterene, does not function solely as a specific antagonist of the mineralocorticoids.[38]

Preliminary communications have described the diuretic activity of the 6-pteridinecarboxamide Wy-5256 (X, $R^1$ = $C_6H_5$, $R^2$ = $R^3$ = 2-methoxyethyl). The renal pharmacology of Wy-5256,[39] and the synthesis and structure-activity relationships of a series of 6-pteridinecarboxamides (X) including Wy-5256,[41,42] now have been published in detail. Wy-5256 has about the same diuretic potency as hydrochlorothiazide in rats and dogs. It probably acts directly on the renal tubule to promote the excretion of nearly equal amounts of sodium and chloride along with some potassium. It is interesting to note that Wy-5256, like furosemide and ethacrynic acid, produces an increase in total renal blood flow.[40] Studies[41,42] on the pteridinecarboxamides (X) have shown that $R^1$ must be

aryl, preferably unsubstituted phenyl, for good oral diuretic activity. In a series where $R^2 = R^3$, maximum activity was reached when these groups are 2-methoxyethyl as in Wy-5256. In a second series where $R^2 = H$, maximum activity was attained in compounds in which $R^3$ is 2-diethylaminoethyl or 2-morpholinoethyl. Activity was lost when the 7-amino group was replaced by hydroxyl.

Pyrazines. N-Amidino-3-amino-6-chloropyrazinecarboxamide (XI)[43] previously was shown to reverse the electrolyte excretion effects of deoxycorticosterone acetate in the adrenalectomized rat and was shown to be a potassium-sparing natriuretic agent in the intact rat and dog.[44]

The preparation and renal electrolyte effects of a series of compounds in which the 5-position of XI has been variously substituted have been reported.[45] Greatly increased activity was found in a group of compounds (XII) which bear a 5-amino substituent. The parent compound, N-amidino-3,5-diamino-6-chloropyrazinecarboxamide (XIIa) is most active, producing a 50% reversal of the DOCA effect in adrenalectomized rats at 2.5 μg./rat. XI produces an equivalent effect at 36 μg./rat.



XI, Y = H

XII, Y = $R^1R^2N$-

XIIa, Y = $NH_2$

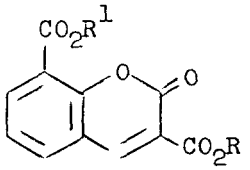
Compounds of type XII in which $R^1$ is H and $R^2$ is straight chain alkyl (methyl through butyl), isopropyl, allyl or cyclopropyl are nearly as potent as XIIa. Similar compounds in which $R^2$ is branched butyl or higher alkyl are considerably less active. The 5-dialkylamino compounds with up to a total of six carbon atoms in $R^1$ and $R^2$ are highly active. The 5-anilino compound (XII, $R^1 = H$, $R^2 = C_6H_5$) is moderately active; the homologous benzyl and phenethyl compounds are weakly active. High potency is retained on introduction of alkyl and aryl substituents at the terminal guanidino nitrogen atoms of XIIa; replacement of the 6-chlorine atom by hydrogen greatly reduces activity. The introduction of halo, hydroxy, alkoxy, mercapto or alkylthio groups into the 5-position gives compounds of greatly reduced activity.

The action of XIIa in reversing the electrolyte effects of aldosterone probably is directly on the distal tubular handling of sodium and potassium rather than through a specific antagonism of the hormone.[46]

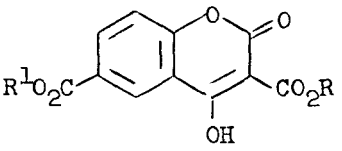
Clinical studies[47-50] show that XIIa·HCl (amiloride hydrochloride) used alone produces a moderate natriuresis and a marked antikaliuresis; it potentiates the natriuretic effects of hydrochlorothiazide and ethacrynic acid in edematous and hypertensive patients while completely reversing the kaliuretic effect of these diuretics.

Other Heterocyclic Compounds. The diuretic properties of 3,6-dicarboxycoumarin and some of its esters have been reported earlier.[51] A new group of coumarincarboxylic acids and their derivatives have been studied[52] in the rat and several members show a similar pattern of

activity, i.e., enhancement of sodium and chloride ion excretion with slight or insignificant effect on potassium excretion. In a series of esters (XIII) of 3,8-dicarboxycoumarin, the 3-ethyl and 3,8-bismethyl esters were the most active saluretic agents (30 mg./kg. orally). A number of lower alkyl esters (XIV) of 3,6-dicarboxy-4-hydroxycoumarin also were active at the same dose and were effective in reversing the electrolyte effects of exogenous aldosterone.

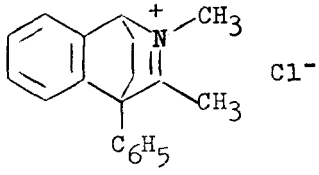


XIII

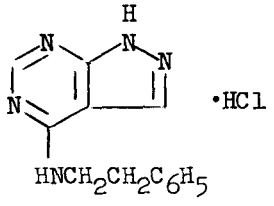


XIV

The cyclic immonium salt, 1,4-dihydro-2,3-dimethyl-4-phenyl-1,4-ethanoisoquinolinium chloride (XV, Su-14074), is a diuretic agent of novel type. It produced a diuretic, natriuretic and chloruretic response in the dog at 5.0 mg./kg. orally.[53] Potassium excretion was decreased. Su-14074 had a similar diuretic and natriuretic effect in the rat at 6.25 mg./kg. orally but was without appreciable effect on potassium excretion.[54] In the dog, Su-14074 reversed the electrolyte effects of injected aldosterone; thus, its action may be due to inhibition of the effect of the endogenous hormone.[53]

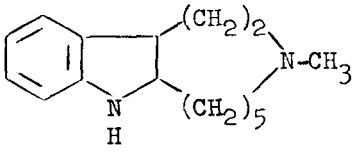


XV

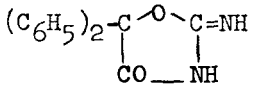


XVI

4-Phenethylamino-1H-pyrazolo[3,4-d]pyrimidine hydrochloride (XVI) was reported[55] to have diuretic and natriuretic activity in rats about equal to that of acetazolamide; however, it appears to be longer acting.



XVII



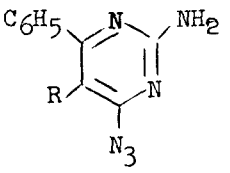
XVIII

An investigation of the biological properties of some azoninoindoles, azecinoindoles and benzazecines showed that several of these bases have significant diuretic and natriuretic activity in rats.[56] The azecinoindole (XVII) is most active, producing a good diuretic response when given orally at 50 mg./kg.

It has been claimed that 5,5-diphenyl-2-imino-4-oxazolidinone (XVIII) produces a prompt, intense diuresis in the rat when administered orally at 20 mg./kg.[57] The volume of urine is increased 5-10 fold; the effect of XVIII on electrolyte excretion was not described.

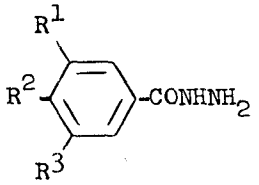
The hypoglycemic and lipolysis-inhibiting compounds, 3,5-dimethylpyrazole and 3,5-dimethylisoxazole, produce a significant diuresis in fasted rats at 100 and 50 mg./kg. i.p. This effect is greatly attenuated in water-loaded rats and is not observed in guinea pigs.[58] The diuretic activity like the hypoglycemic activity of these compounds appears to be species-specific.

The azidopyrimidines (XIX), 5-ethoxyethyl- and 5-propargyl derivatives of 2-amino-4-azido-6-phenylpyrimidine, reverse the oliguria produced by ADH in the alcohol-saline loaded rat[59] at doses less than 1 mg./kg. This action is the result of a nonspecific antagonism to the hormone since both compounds produce diuresis and saluresis in the absence of injected ADH. Studies of the diuretic action of SC-16102 in the dog also have been reported.[60]



XIX, R = $C_2H_5OCH_2CH_2$- (SC-16102)
$CH{\equiv}CCH_2$- (SC-16100)

Hydrazides. A series of hydrazides and sulfhydrazides were tested orally as diuretics in mice at 50 mg./kg. Two compounds, XX and XXI, were as active as chlorothiazide at 5 mg./kg. Three other compounds, XXII, XXIII and propionhydrazide, were somewhat less active.[61]

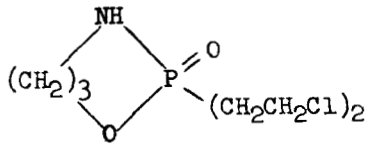


XX, $R^1$ = $CH_3O$; $R^2$,$R^3$ = H
XXI, $R^2$ = HO; $R^1$,$R^3$ = H
XXII, $R^1$ = HO; $R^2$,$R^3$ = H
XXIII, $R^1$,$R^2$,$R^3$ = HO

Hormonal Factors. A number of publications have centered on the mode of action and the inhibition of the antidiuretic hormone (ADH, vasopressin). It was shown that ADH in the rat may regularly cause diuresis as well as the generally observed antidiuresis.[62] This biphasic action is dose-dependent, i.e., small doses of the hormone injected or infused

produce diuresis; larger doses produce antidiuresis. An analogous dual action of oxytocin on water excretion was also reported.[63] Studies in human subjects support the previous hypothesis that cyclic adenosine 3',5'-monophosphate is the intracellular mediator of the antidiuretic action of ADH. Given intravenously, the nucleotide decreased urinary volume and raised osmolarity in normal subjects given an excess water load; in a patient with diabetes insipidus, it produced a response exactly like ADH (pitressin).[64]

Cyclophosphamide (XXIV) (50 and 100 mg./kg.) and its metabolite 2,2'-dichlorodiethylamine, were found to produce a water diuresis in rats and dogs.[65] Evidence was presented that these compounds act by inhibiting the action of ADH. A similar effect of 5,5-diphenylhydantoin in man at the high i.v. dose of 500 mg. was reported.[66]




XXIV

The interesting observation that the analgesic acetaminophen (N-acetyl-p-aminophenol) accelerates net water flow across the isolated toad bladder led to its trial in patients with diabetes insipidus.[67] A single dose (1.2 to 2.4 g.) produced a marked fall in urinary excretion and a rise in osmolality. It was suggested that acetaminophen may be a useful substitute for vasopressin in diabetes insipidus.

The complex effects of aldosterone infusions on the renal response to angiotensin in normal and edematous subjects have been studied.[68]

Diuretics from Plant Sources. Reports of diuretic activity in naturally-occurring substances or their extracts have been abundant in the past and they are always difficult to evaluate. Recently, diuretic properties have been described for extracts of birch leaves[69] (Betula verrucosa), celery[70] (Apium graveolens) and the Indian plants, Boerhaavia repens and Boerhaavia rependa.[71]

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# Chapter 8. Angina Pectoris and Antianginal Agents

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It is generally agreed that the pain associated with angina pectoris is due to a relative hypoxia of the myocardium. Attacks of angina can be precipitated by increasing the heart's oxygen demand, or by decreasing its supply. Emotional factors play a major role in increasing myocardial oxygen requirements by increasing cardiac sympathetic nervous activity.[1]

The heart is a curious organ. In contrast to skeletal muscle, myocardial metabolism is entirely aerobic, there being little or no conversion of glucose or glycogen to lactic acid. In fact, heart muscle extracts lactic acid from the blood, converting it to pyruvic acid.[2] Production of lactic acid by the myocardium has been observed during exercise of patients with coronary artery disease. Lactic acid production is most frequently observed in extensively diseased hearts, and is usually associacted with disease of the left anterior descending coronary artery.[3] Skeletal muscle extracts about 22 percent of the available blood oxygen, while extraction in the heart runs about 70%.[4] This high arterio-venous oxygen difference requires that changes in myocardial oxygen supply be dependent on modifications in coronary blood flow (CBF).

Drug therapy of angina pectoris has traditionally involved the use of agents believed to be coronary vasodilators in an effort to increase CBF. Some agents which produce a marked increase in CBF in the normal subject fail to effect either an increase in CBF or relief of pain in patients with angina pectoris. Failure of purported coronary vasodilators to relieve angina may be due to a number of factors. The myocardium possesses an extremely efficient auto-regulatory system, the strongest stimulus for vasodilation being hypoxia. In order to produce a therapeutic degree of vasodilation in the diseased heart, a drug must produce a greater degree of vasodilation than the hypoxia itself. Gorlin has concluded that the failure of vasodilators to increase CBF in anginal patients might be due to a hypoxia-produced exhaustion of the coronary reserve for further vasodilation.[5] McGregor and Fam have pointed out that this does not exclude the effect of these drugs on collateral flow. These authors accept the theory of an auto-regulatory mechanism which adjusts blood flow to metabolic need. This adjustment depends on the presence of a metabolite in an arteriole in close contact to the myocardial cell. This mechanism regulates the flow of blood at the site of oxygen utilization and should not be disturbed. In the ischemic heart, the flow of blood to the auto-regulated arterioles is insufficient. The useful antianginal agent should exert its effect by increasing the blood supply to the arterioles by acting on the large conductive and collateral vessels. McGregor and Fam assert that an agent which increases CBF by interfering with the auto-regulatory function

will allow those arterioles which are already receiving adequate blood to further divert blood away from ischemic areas and into healthy ones.[6]

During the last few years there has been a de-emphasis of the anatomical considerations associated with angina pectoris and an increasing concern of a possible upset of auto-regulation or of sympathetic stimulation in the heart. Berne suggested the role of adenosine nucleotide breakdown products in the auto-regulation of CBF.[7] In the presence of a deaminase inhibitor, adenosine has been observed in the perfusate of isolated cat and guinea pig hearts subjected to anoxia. The increase in CBF during graded hypoxia has been found to be proportional to the sum of adenosine, inosine, and hypoxanthine released from myocardial tissue.[8] The possible involvement of plasma kinins in the auto-regulation of CBF has been suggested. Bradykinin, kallidin, or eledoisin were found to increase coronary sinus outflow, coronary sinus oxygen tension, and stroke flow without affecting blood pressure or heart rate. The effects of these kinins were attributed to coronary vasodilation by a direct action on the myocardial vessels.[9] Gorlin has suggested that plasma kinins, released from ischemic or inflamed tissue, may be pain producing substances.[10] It should be pointed out that it is not known whether or not these agents are released into the circulation during attacks of angina pectoris.

In the course of their work on a canine heart model for angina, Szentivanyi and co-workers claim to have found evidence for the existence of a substance, hyperemin, which regulates coronary vessel responsiveness to metabolic requirements. In the absence of hyperemin, vasodilation could no longer be produced by metabolic stimuli or by adrenalin. Administration of hyperemin from a donor heart reestablished normal responses.[11] Presumably, angina pectoris may involve a lack or insufficiency of a hyperemin type material in the human heart.

The hemodynamics associated with attacks of angina pectoris have been extensively studied. Diamond reported elevated left ventricular end diastolic pressures during exertional angina.[12] Roughgarden noticed an increased systemic and pulmonary blood pressure during attacks of exertional and spontaneous angina.[13,14] Gorlin and his associates reported a maximal increase in left ventricular end diastolic pressure of 3mm Hg. during exertional angina and no pressure change when angina followed the administration of isoproterenol.[15] Parker observed slight increases in left ventricular end diastolic pressure to precede the onset of exertional angina. However, no correlation was found between the onset of pain and the development of pressure changes.[16] McGregor's group found no difference in the hemodynamics of anginal patients from normal subjects, even though the patients were experiencing anginal pain. The response of both groups to sublingual administration of nitroglycerin, either at rest or ten minutes before exercise, was similar.[17] Roughgarden maintains that an anginal attack is accompanied by an increase in pressure and that relief from the attack, whether spontaneous or as a result of nitroglycerin treatment, is accompanied by a drop in blood pressure towards the pre-attack level.[14]

The evaluation of possible antianginal agents has been limited to

determination of CBF changes. The methodology of screening antianginal compounds has been reviewed.[4,18,19,20] Rowe criticized available methods of evaluation on the basis that they give no information concerning the distribution of blood flow within the myocardium or the effects on arteriovenous communications.[20] It is quite likely that differences in distribution of CBF rather than total flow are important.

A major difficulty with transferring results obtained in the laboratory to the clinic is the fact that these studies are usually carried out in animals with normal circulation, or other situations which differ greatly from those found in the diseased heart.

There have been many efforts to screen antianginal drugs using experimentally induced coronary insufficiency. Szentivanyi and Juhasz-Nagy have attempted to produce an animal model to simulate angina pectoris. A functional coronary rigidity resembling that found in anginal subjects was induced in the dog heart by prolonged hypoxia, bilateral stellectomy, or by intracoronary injection of hexamethonium or dibenamine. These workers claim that papaverine and prenylamine (I) cause vasodilation in the state of functional coronary rigidity. Intracoronary injection of papaverine produced an increased flow but did not restore the hyperemic response to brief reduction in coronary flow.[21] Unfortunately, these authors did not report the effects of nitrates on their model. The use of pituitrin induced coronary vasoconstriction has recently been reinvestigated by Papp and Szekeres. Injection of pituitrin produced coronary insufficiency in the conscious rabbit as measured by the elevation in the T wave in the ECG. The lowest dosage of drug capable of normalizing the T wave amplitude in 50% of the test animals, or the percentage decrease in the T wave produced by different dosages of the drugs, was claimed to be more sensitive for drug evaluation than results obtained by inhibition of pituitrin induced vasoconstriction.[22] In all attempts to produce experimental coronary ischemia, the question remains as to how closely the experimental procedure resembles the clinical situation.

The clinical evaluation of antianginal drugs has been recently reviewed.[23] Russek has criticized the techniques used in the evaluation of antianginal drugs. The use of a double blind technique is no guarantee that the results are infallible. According to Russek, many of these studies involve an experimental design in which the drug is administered without regard to its known pharmacological action and no effort is made to synchronize the drug effect with the periodicity of anginal attacks.[24]

Wendkos has proposed an "Orthostatic Electrocardiographic Index" in an attempt to demonstrate nitrate activity.[25] This study is hardly conclusive as it was carried out on four schizophrenics without demonstrable organic heart disease. Wendkos recently reported that nitroglycerin and isosorbide dinitrate abolished ECG signs of ischemia provoked by i.v. administration of ergot alkaloids to anginal patients.[26]

The use of ECG changes in evaluating antianginal drugs has been widely criticized. The ECG gives only indirect information concerning the state

and adequacy of the coronary circulation.[5] Abrahamsen and Kiil maintain that ST changes may not reflect myocardial blood supply, as disturbances in CBF can be reversed without changes in the ECG.[27]

The lack of understanding of angina pectoris is reflected in the wide variety of drugs used in the management of this syndrome. In a recent review, Master listed 70 different medications including narcotics, vasodilators, tranquilizers, antispasmotics, anticoagulants, hypometabolic agents, estrogens, hypercholesterolemia antagonists, MAO inhibitors, and vitamins.[23]

Despite criticism of its efficacy and mode of action, nitroglycerin remains the most widely used drug in anginal therapy.[28,29] There has been a growing realization that nitroglycerin may exert its therapeutic effect through a number of mechanisms. It is well known that nitroglycerin increases CBF in normal individuals. Using nitrous oxide desaturation techniques to determine CBF, Gorlin found sublingual nitroglycerin to decrease CBF by 16% in patients with angina pectoris. Gorlin suggests that the beneficial effects of nitroglycerin may be the result of decreased myocardial contractility.[5] The use of nitrous oxide techniques has been criticized and they have been replaced by methods based on radioactive isotopes.[20] Bing's group, using $Rb^{84}$ in anginal patients, found a 13% decrease in CBF 2-8 minutes after sublingual nitroglycerin administration, and a 5% decrease after 8-15 minutes. Bing reports similar decreases in CBF for iproveratril (II) and carbochromene (III).[30] Measurements of CBF using $Xe^{133}$ have shown nitroglycerin to have a biphasic response. Rees reported that in dogs nitroglycerin produced an increase in left ventricular efficiency, as well as an immediate rise in CBF which was greatest at the end of injection, fell away at 3 minutes and reached a minimum at 6 minutes, then returned to normal. Rees suggested that the effect of nitroglycerin on the coronary vessels is brief and restricted to the first phase. The subsequent increase in coronary resistance might then be an auto-regulatory effect due to reduced myocardial oxygen requirements.[31] Bernstein, using $Xe^{133}$ techniques, found intracoronary injection of nitroglycerin to produce a biphasic response in man as well as dogs. In patients with angina pectoris, there was a 38.5% increase in CBF ½ minute after administration of nitroglycerin. At 4-6 minutes the increase in flow was only 4.8%. This brief increase in CBF could bring about relief of pain by removal of metabolites from ischemic areas of the myocardium. Bernstein suggests that systemic effects predominate in the second phase of nitroglycerin response to give decreased arterial pressure, cardiac output, and decreased coronary vascular resistance. Because of the drop in atrial pressure, coronary flow falls below control levels. The prophylactic and sustained actions of nitroglycerin could be due to a lowering of myocardial metabolism.[32] Bernstein's results are open to criticism because of the use of intracoronary injections of relatively high concentrations of nitroglycerin.

McGregor's group maintains that retrograde flow into an ischemic area may be increased for twenty minutes following nitroglycerin. The action of nitroglycerin would then be due to a redistribution of blood within the

myocardium, accompanied by a reduced energy expenditure.[6] Najmi found nitroglycerin to reduce pulmonary artery pressure, total pulmonary resistance and right ventricular work. Najmi suggested that pooling of blood in the peripheral venous, or pulmonary vascular systems, would result in a decreased heart size with concomitant reduction in cardiac work.[33] It seems unlikely that this rationale alone would explain the action of nitroglycerin, since vasodilation and pooling of venous blood could be accomplished by vasodilators, which are ineffective in relieving anginal pain.

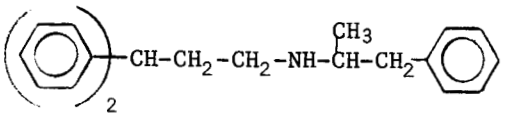
Needleman attempted to correlate the antianginal activity of nitrates with their ability to produce loss of respiratory control in mitochondria. He suggests that nitroglycerin exerts its effect by reacting with SH groups within the mitochondria, thereby causing an uncoupling of phosphorylation.[34]

Russek has found isosorbide dinitrate and pentaerythritol tetranitrate to produce a significant improvement in exercise tolerance as well as exercise ECG tests.[35] A wide variety of nitrate esters have been found to provide relief of angina pectoris. There is no information, however, to suggest a difference in the basic pharmacological action of these compounds. Such differences are presumably due to differences in absorption and rates of metabolism.[36,37,38]

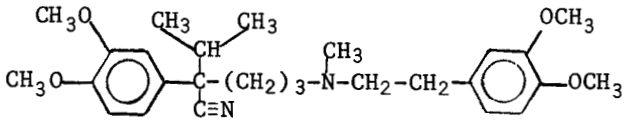
Dipyridamole is a potent coronary vasodilator. Despite the fact that it causes a substantial increase in CBF, its value for the acute relief or prevention of angina pectoris has been seriously questioned. Considerable effort has been expended in the study of this drug. Deutlicke reports dipyridamole to inhibit adenosine deaminase which results in an accumulation of adenosine. Since adenosine is a potent vasodilator, there is an increase in CBF.[39] Emmons found dipyridamole to inhibit platelet agglutination and proposed that dipyridamole prevents the uptake of adenosine by red blood cells, thereby delaying its deamination.[40] Stafford feels that dipyridamole inhibition of adenosine deaminase is unlikely, as high concentrations are necessary to produce inhibition of deaminase from intestinal mucosa. Much lower concentrations of dipyridamole potentiate the action of adenosine on myocardial tissue. Stafford proposes that dipyridamole prevents the uptake of adenosine by myocardial tissue or red blood cells.[41] Schmidt reported dipyridamole produces increased myocardial collateral circulation in dogs.[42] McGregor found dipyridamole to increase cardiac output and ventricular minute work as a result of increased stroke volume.[43] McGregor maintains that the increased CBF is a result of an increase in the rate of capillary flow[44] rather than a result of shunting through other pathways as suggested by Winbury.[45]

Prenylamine (I) was introduced in Europe as a coronary vasodilator which reduces the noradrenaline and serotonin content of the heart. Initial double blind studies have shown a decrease in the pain of angina pectoris. Results using objective methods, based on ECG changes and Masters Two-Step techniques, have been equivocal. Ito found prenylamine, dipyridamole, and 7-chloroethyltheophylline to increase diastolic pressure as well as CBF. Ito states that factors other than increased diastolic

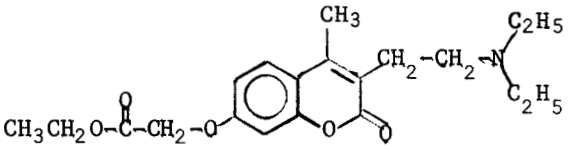
pressure are associated with increased CBF and suggested dipyridamole dilates coronary arteries even in coronary sclerotic patients.[46] Jacobsson found no difference between the effects of oxyethyltheophylline, prenylamine, or placebo in the relief of subjective or ECG signs of ischemia.[47]



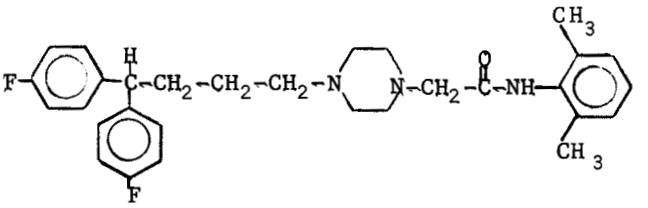
Prenylamine
Elocor
Hostaginan
Segontin
Synadrin
I



Iproveratril
Isoptin
II



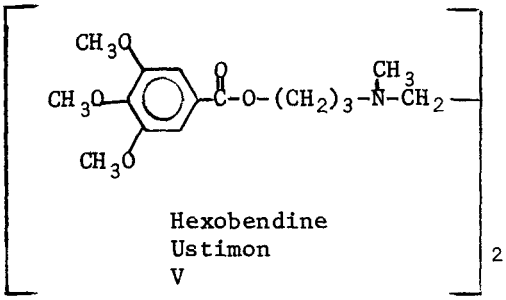
Carbochromene
Intensain
III



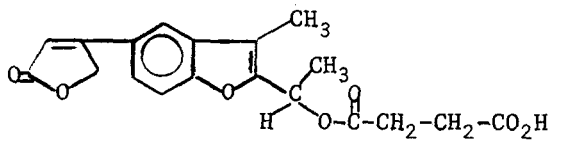
Lidoflazine
IV

A number of compounds have been reported which have coronary dilator properties or other activities which have culminated in their clinical trial for use in angina pectoris. Lidoflazine (IV) is claimed to be a specific, long acting coronary vasodilator. This agent appears to reduce coronary vascular resistance in dogs and causes an increase in coronary venous $pO_2$.[48]

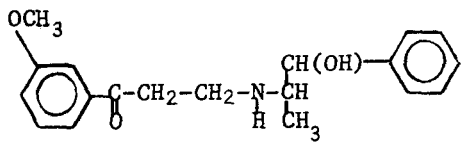
A study of iproveratril (II), carbochromene (III), hexobendine (V), and dipyridamole indicates that these long acting coronary vasodilators probably do not exert their action by an adrenergic mechanism. Only in the case of carbochromene was the arterio-venous oxygen difference affected by prior reserpinization. The dose-related activity in reserpinized dogs suggests that the primary effect of carbochromene is not adrenergic.[49] Carbochromene has been reported to increase CBF by prolonging the action of adenosine on the coronary arteries.[30] Iproveratril and carbochromene have been clinically available in Europe for some time. Iproveratril is currently being investigated in the United States.[50] Clinical trials of hexobendine have not been reported but the compound appears to be headed for the clinic.[51]



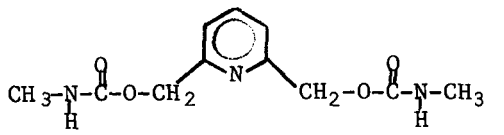
Hexobendine
Ustimon
V



Benfurodil
VI



Ildamen
VII



Pyridinolcarbamate
Anginin
VIII

Benfurodil (VI) has been claimed to be a cardiotonic-vasodilator, which has clinical value in angina pectoris. However, no data is available.[52] Ildamen (VII) has recently been placed on the European market as an antianginal agent. Ildamen has a positive inotropic activity and appears to be a stimulator of beta adrenergic receptors.[53] Pyridinolcarbamate (VIII), a bradykinin antagonist, has been shown to be an anti-arteriosclerotic agent in rabbits, and is claimed to be beneficial in

angina pectoris, as well as in reopening occluded arterial segments in patients suffering from arteriosclerosis obliterans.[54,55]

A direct relationship between the catecholamines and angina pectoris has been known for some time. Raab proposed that attacks of angina pectoris result from a coincidence of two basic factors: 1. A predisposing element of impaired coronary dilatability due to sclerosis, and 2. A cardiotoxic trigger involving a temporary influx into the heart of catecholamines. Raab also suggested that a direct action of catecholamines on the coronary vessel may be involved in the genesis of arteriosclerosis.[56] It is generally accepted that an overexcitability in the sympathetic nervous system exists in a substantial number of individuals with angina pectoris. Valori recently reported that urinary excretion of norepinephrine and epinephrine was greatly exaggerated following myocardial infarction.[57] Nestel has shown that compared with normal men, those with angina pectoris secrete abnormally large amounts of norepinephrine and similar amounts of epinephrine in response to emotional stress.[58]

Epinephrine and norepinephrine produce an increase in coronary blood flow. Nevertheless, these agents have a deleterious effect on patients with angina pectoris because the increased CBF is insufficient to meet an increased oxygen requirement, which is a result of increased blood pressure, heart rate, and myocardial contractile force.

Ahlquist has attempted to explain the actions of the catecholamines in terms of two different types of adrenergic receptors.[59] According to Ahlquist's nomenclature, alpha receptors generally mediate vasoconstriction and excitatory functions, while stimulation of the beta receptors produces vasodilation and other responses associated with smooth muscle relaxation.[60] Beta receptors are believed to mediate the chronotropic and inotropic action of catecholamines. Epinephrine is active on both alpha and beta receptors while the action of norepinephrine is primarily on the alpha sites. Isoproterenol has little action on alpha sites, being a potent stimulator of beta receptors.

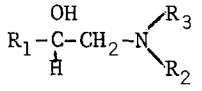
There has been a great deal of interest in the nature of coronary sympathetic stimulation. Braunwald's group observed coronary vasodilation after the intracoronary injection of isoproterenol and concluded that beta receptors are present in the coronary circulation.[61] Gilmore has recently reported the presence of both alpha and beta receptors within the coronary vasculature.[62] Govier found both alpha and beta receptors as evidenced by changes in atrial refractory period.[63] Gaal and his associates have suggested that both alpha and beta receptors are present in the coronary vessels and that normally the beta response predominates. After blockade of the beta receptors, administration of epinephrine or norepinephrine results in stimulation of only alpha receptors which results in coronary vasoconstriction.[64] Zuberbuhler and Bohr studied the response of smooth muscle from various sized coronary arteries and concluded that alpha and beta receptors are present in the larger arteries, but that beta receptors predominate in the smaller vessels.[65] Takenaka has observed similar effects in the isolated perfused dog heart.[66] Braunwald proposed that

both alpha and beta receptors are present in the coronary arteries but only beta receptors are found in the myocardium.[67]

By electrical stimulation of the stellate and caudal cervical ganglia, Szentivanyi observed simultaneous dilation in one region of the myocardium with constriction in another. Szentivanyi concluded that the myocardium and coronary arteries are supplied by different sympathetic pathways.[68] It has been suggested that the vasodilation observed on stimulation of sympathetic fibers is a result of a sympathetic cholinergic innervation.[69] In a recent study, Feigl stimulated the stellate ganglion and hypothalamus and found no evidence of cholinergic mediated coronary vasodilation.[70] Information obtained by sympathetic stimulation of the coronary circulation has been recently criticized. The distribution of alpha and beta receptors within the coronary vasculature may vary with species and physiological state of the animal. Different experimental approaches may favor stimulation of either alpha or beta receptors. Other factors may be involved such as dose or time dependent actions of catecholamines on different receptors.[71]

Because of their possible role in angina pectoris, the chronotropic, inotropic, and oxygen wasting effects of the catecholamines have been greatly explored. Braunwald maintains that the decreased efficiency in myocardial oxygen utilization produced by catecholamines is a consequence of increased myocardial tension.[67] However, the consensus of opinion seems to be that these effects are due to changes in myocardial metabolism involving stimulation of the beta receptors.[72] Interaction with the beta receptor is believed to result in the formation of adenyl cyclase which catalyzes the formation of 3', 5'-cyclic adenosine monophosphate. Cyclic 3', 5'-AMP acts as a secondary mediator by increasing glycolysis and lypolysis as well as increasing cardiac contractility.[73,74,75] Svedmyr recently demonstrated that blockade of beta receptors with MJ-1999 prevents the myocardial stimulation, hyperglycemic, and free fatty acid and lactate mobilizing effects of catecholamines.[76]

In view of the known actions of catecholamines on the myocardium and from the observation that the individual with angina pectoris has a hyperactive sympathetic system, it is rationalized that blockade of the beta adrenergic receptors may benefit the anginal patient. There are a number of agents which produce a selective blockade of the beta adrenergic receptor, their structure activity relationships have been reviewed.[77,78,79]



In general, beta adrenergic blocking agents are ethanol amine derivatives. Dichloroisoproterenol ($R_1$=3,4-dichlorophenyl, $R_2$=isopropyl, $R_3$=H), was the first agent to display selective beta blocking activity. However, it also possesses an intrinsic beta stimulatory action. Pronethalol, ($R_1$=1-naphthyl, $R_2$=isopropyl, $R_3$=H) was found to have a beneficial effect in anginal patients by beta blockade which reduced myocardial oxygen requirements.[80] Pronethalol has some stimulatory action on the beta receptor and was withdrawn when it was found to produce thymic lymphosar-

coma in mice.[81] A large number of phenethanol amine derivatives block the beta receptor. INPEA ($R_1$=p-nitrophenyl, $R_2$=isopropyl, $R_3$=H) selectively blocks beta sites without influencing alpha receptors.[82] Dimethylisopropylmethoxamine ($R_1$=2,4-dimethylphenyl, $R_2$=isopropyl, $R_3$=$CH_3$)[83] and t-butylmethoxamine, ($R_1$=2,4-dimethoxyphenyl, $R_2$=t-butyl, $R_3$=$CH_3$)[84] have been reported to produce a selective blockade of some, but not all, beta receptors. MJ-1999 ($R_1$=p-methanesulfonamidophenyl, $R_2$=isopropyl, $R_3$=H) has been reported to be a selective blocker of beta receptors.[76]

Propranolol ($R_1$=1-naphthyloxy, $R_2$=isopropyl, $R_3$=H) has elicited a great deal of attention in the treatment of angina pectoris.[85] Harris reported that propranolol produced a decrease in heart rate, cardiac output, stroke volume, ejection time index, and significant increases in atrial and mean right atrial pressures. Propranolol blocks the effects of isoproterenol and causes epinephrine reversal.[86] The reversal of epinephrine as well as increases in atrial pressures is probably due to an unmasking of the less significant stimulatory effect of the alpha receptors. Working with dogs, Rowe's group found propranolol to decrease body oxygen consumption, cardiac output, CBF, and left and right ventricular work. Total peripheral, pulmonary and coronary vascular resistances were increased.[87] Nayler recently reported that in the anesthesized dog, propranolol produced a 29% decrease in CBF as well as a decrease in myocardial oxygen consumption.[88] Sowton and Hamer, in anginal patients, found that propranolol reduced cardiac output 20% both at rest and on exercise. Stroke volume was reduced 15% at rest and 5% during exercise. Heart rate fell 10% at rest and 15% during exercise. Left ventricular work was reduced by 21% at rest and 24% on exercise. These changes were accompanied by significant decreases in oxygen uptake.[89] Chamberlain found propranolol increased heart size at rest and during exercise in normal subjects.[90]

In double blind studies, Gorlin's group found an 81% improvement in anginal patients when treated with propranolol. Propranolol produced a decrease in heart rate, end diastolic ventricular volume and a fall in left ventricular systolic mean pressure. Mechanical efficiency was not changed by propranolol but oxygen consumption decreased 25%. Gorlin notes that CBF decreases more than oxygen consumption suggesting some coronary vasoconstriction, probably a result of alpha stimulation.[91] Ginn and Orgain, in a double blind study of 18 patients, found an 83% improvement in anginal pain frequency.[92] Hamer found propranolol to increase exercise tolerance in anginal subjects and suggested that this is obtained at the expense of a reduction in myocardial contractility and in peripheral blood flow.[93] Rabkin has found improvement in anginal patients and suggested there is a carry over effect after discontinuing the drug.[94] Rabkin's observation is interesting as propranolol is known to have a relatively short half-life.[81]

Exercise tolerence has been significantly increased by a combination of propranolol and nitroglycerin, suggesting a possible synergistic effect.[95] Russek recently reported that a combination therapy of pro-

pranolol and nitroglycerin resulted in improvement in the frequency of anginal attacks and ECG signs of ischemia. Russek maintains that nitroglycerin compensates for the vasoconstriction action of propranolol.[96]

While many investigators have shown propranolol to produce an improvement in exercise tolerance or consumption of nitroglycerin tablets, there is no work demonstrating improvement in ECG signs of ischemia. Gillam and Prichard studied ECG signs during exertional angina of 7 patients treated with propranolol. Four patients showed a greater degree of ST depression while 3 patients showed less.[97] Fisch has criticized the use of beta adrenergic blocking agents in angina pectoris since these agents should produce an *in vivo* coronary vasoconstriction, an effect which is in contradiction to the customary use of vasodilators in angina.[98] It is of interest that beta adrenergic blockade has not proved effective in preventing attacks of angina of an emotional origin.[99]

Snow reported a decreased mortality rate following myocardial infarction when patients were treated with propranolol.[100] The increased survival rate following infarction was attributed to a prevention of cardial arrhythmias by beta adrenergic blockade.[101,102,103] More recent, carefully controlled, double blind studies have failed to confirm Snow's findings of increased survival.[104,105] It has been pointed out that beta blocking agents should be restricted to patients without overt signs of myocardial decompensation. Blockade of the sympathetic nervous system in an impaired heart removes the drive necessary to maintain cardiac function.[106]

The possibility of treating angina pectoris with beta adrenergic blocking agents has opened new avenues of research, particularly in the area of cardiovascular sympathetic innervation. The future role of these agents in the therapy of the anginal syndrome is a question which is yet to be fully answered. It is hoped that mistakes made in past investigations of antianginal agents will not be repeated in this area.

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# Chapter 9. Pulmonary and Antiallergy Agents.

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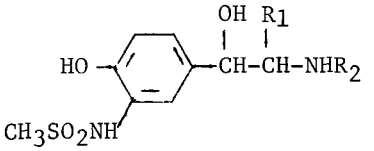
The year's most significant developments in the fields considered to be within the scope of this chapter[1] occurred in areas associated with chronic obstructive lung disease. For the most part, these appeared as reports summarizing and clarifying older thinking regarding cause and effect relationships, or as studies which suggest new approaches to be explored. Most of the reported drug developments can be identified as arising in a fairly straightforward manner from earlier work.

Obstructive Lung Disease.--Carabasi[2] briefly summarized some important differences between bronchial asthma, chronic bronchitis, and pulmonary emphysema, which are the presently recognized major categories of chronic obstructive lung disease. While it is obvious that these diseases have many common features and that methods of treatment will continue to overlap substantially, such distinctions are of great value because they are an essential first step in identifying new approaches that can be logically pursued with specific methodology. Thus, bronchial asthma is viewed as an allergic phenomenon associated with bronchial and tracheal hypersensitivity to challenge from a variety of stimuli[2-4] --a view that is consistent with the fact that corticosteroids continue to occupy a predominant position in its treatment.[5,6] It follows that the emergence of significant improvements in the primary treatment of bronchial asthma should continue to depend on the realization of a clearer understanding of allergy. Chronic bronchitis, on the other hand, is believed to be associated with airways obstruction arising from abnormalities in mucous secretions.[2,7,8] This suggests that better knowledge of the systems responsible for mucus production and transport should lead to the evolution of drugs more effective than the presently available mucolytics and expectorants.

In contrast with the above diagnostic categories, in which reversible changes are often recognized soon enough to respond to appropriate therapy, emphysema is presently thought to result from the irreversible destruction of lung tissue.[2] Consequently, present methods for handling emphysema are usually palliative or prophylactic, and the development of rational treatment awaits identification and understanding of the underlying disorder. Since the onset of emphysema appears to be associated with repeated injury to lung tissue by inhaled irritants, by infection, or by other factors that are as yet unrecognized,[2,9] it is reasonable to seek experimental models in animals subjected to a comparable challenge. Rats exposed to sulfur dioxide,[10] or dogs to cigarette smoke,[11] over extended periods of time, and newborn piglets subjected to prolonged hypoxia[12] were reported to develop emphysema-like conditions. It remains to be shown that such models are an accurate reflection of the human disease and, in their present state of development, it seems likely that they

will be more useful for identifying specific factors to be explored in detail than as routine tools to be used in drug design. Reports such as those that associate familial emphysema with serum alpha$_1$-antitrypsin deficiency[13] may provide useful clues to understanding the etiology of the tissue destruction characteristic of this disease.

Bronchodilators.--Bronchospasm of varying degree is present in all types of obstructive lung disease.[8,14] This accounts for the widespread symptomatic use of bronchodilators, and they should continue to occupy an important therapeutic position, even if there should be dramatic improvements in primary therapy. Despite this, there were few significant developments in this area during 1966 and much of the following is a direct extension of matters discussed last year.[1] Recent clinical reports, which summarize many earlier studies, indicate that metaproterenol may have significant advantage over the standard sympathomimetic bronchodilator, isoproterenol, in terms of side effects and oral absorption.[15] Several papers reemphasized the suitability of tracheal muscle preparations as an in vitro test for bronchodilator action.[16] Lands and coworkers[17] extended their studies of differences in β-adrenergic receptors at various physiological sites, and a possibly related difference between bronchial and cardiovascular β-receptors was suggested in clinical studies with metaproterenol and the β-receptor blocking agents Kö-592 and propranolol.[3,18] Studies with the methanesulfonamido analogs of catechol amines have shown two, MJ-1992 and MJ-1987, to have good bronchodilator action.[19] Bronchodilator activity, along with other properties believed to be desirable in agents for treating cor pulmonale, was reported for the two non-sympathomimetic structures CIBA 31531-Ba[20] and MJ-1988.[21]

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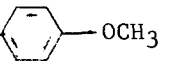
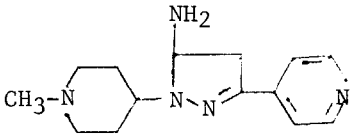
| MJ-1987 | $CH_3$ | $-(CH_2)_2$  |



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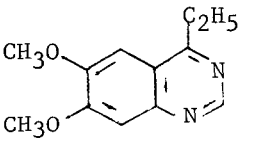



MJ-1988

Antitussives and Mucolytics.--Chalmers,[22] in a short review of currently available cough suppressants, emphasized that antitussive action mediated solely through inhibition of the cough reflex is generally undesirable in chronic obstructive lung disease, and that the rational approach to treating persistent coughing is to eliminate the causative irritation. It follows from this position that traditional cough suppressants,[23-27] as well as methods which conventionally involve comparisons with codeine[28] in situations where coughing is acutely induced,[29] are likely to be of limited value for chronic use.

More significant antitussive action should be expected from drugs that act directly on the mucociliary system, which is more closely associated with the cause of the cough. The proceedings of symposia[30-32] on mucus production, transport, and function in various organs suggest an acceleration of research in this area. On the other hand, the scant space devoted to respiratory tract secretions in Gottschalk's monograph on glycoproteins[33] is indicative of an urgent need for additional factual information.

The respiratory tract mucociliary system is responsible for the removal of inhaled foreign substances, and serves an important emollient and protective function;[34] deficiencies in these functions would be expected to lead to obstruction or to cough-producing irritation which may be further aggravated by defects in mucus production. Hence, potentially useful drug actions include, (1) alterations in the quantity and/or quality of mucus produced, (2) adjustments in the quality of mucus by direct physical or chemical action, and (3) regulation of ciliary action which, assisted by the cough reflex, is responsible for moving mucus and attendant impurities outward against the force of gravity.[34-36] Little is known about the physiological control of mucus secretion. There is evidence for the involvement of serotonin in the control of gastric mucus secretion (and against adrenergic or cholinergic control),[37] and the fact that gastric irritation can produce reflex secretion of mucus in the bronchioles,[22] suggests common control mechanisms in both organs. Even less is known about the physiologic control of mammalian ciliary action;[38] innervation of ciliary cells cannot be demonstrated[35] and no neurohumoral control mechanisms are presently recognized,[39] although Spock reported that ciliary action in tissue cultures of human nasal polyps is mildly stimulated by acetylcholine.[40] (The same paper reports profound stimulation of cilia under these conditions by ATP.) Cilia may be responsive to changes in pH[40] or ionic strength,[36] and it was suggested that ciliary beat frequency is a function of the amount and viscosity of tracheal secretions.[41]

Perhaps as a result of this lack of understanding, efforts generally centered on examining the functional properties of mucous secretions, and discovering means to alter them. Methods were reported for the objective measurement of mucous flow and viscosity, which attempt to overcome difficulties associated with the non-homogeneous nature of most samples of bronchial mucus and with the measurement of such properties in situ.[36, 42-45] N-acetylcysteine, to judge from the number of favorable clinical reports, has emerged as the most useful, locally-acting mucolytic agent presently in common usage.[46] Its mucolytic action is presumed to depend on sulfhydryl-disulfide interchange reactions which reduce the molecular size of mucous glycoproteins, thus lowering the viscosity of secreted mucus. A related approach, the local application of 2-4 M solutions of urea, almost certainly depends upon the well-known ability of this agent to alter higher order structure and solubilize biological macromolecules in aqueous solution.[47]

The systemic drugs Bisolvon® and pimetine, mentioned last year,[1]

continued to attract attention. Favorable clinical reports from trials with the former, administered orally, parenterally, or by aerosol, have now reached several thousand cases in the European literature--its action being variably described as antitussive, mucolytic, or expectorant. Studies in rabbits show that the drug, at 1 mg/kg orally, increases the volume of respiratory tract fluid, which is accompanied by a decrease in the concentration of solids and probable decreases in the concentration of glycoproteins.[48] These effects appear to parallel those seen in man and suggest that the drug should be classified an expectorant. This expectorant action was inhibited by atropine, but the very large doses required (20-40 mg/kg) do not appear to support the proposition that Bisolvon® acts through a specific cholinergic mechanism. It was pointed out that similar actions have been reported with emetine and the ipecac alkaloids, as well as with pilocarpine. Electron microscopic studies in rats showed that Bisolvon® induces changes in the secretory cells of trachea and upper bronchi, and it was suggested that secretion of mucolytic enzymes might be stimulated by the drug.[49] Pimetine, on the other hand, was reported to be clinically superior to aminophylline in reducing sputum volume, equivalent in relieving cough, and inferior in improving lung function. Its airways-clearing effect, termed "broncho-perviant", was ascribed to decreases in volume, viscosity, and adhesiveness of abnormal respiratory tract secretions.[50] Present evidence, therefore, does not suggest that these two agents act by the same mechanism, although additional comparative studies to establish the point are clearly desirable.

Respiratory Stimulants.--Therapeutic measures to overcome ventilatory deficiencies and to reduce the blood carbon dioxide levels in obstructive lung disease were thoroughly discussed by several authors.[51] The use of currently available respiratory analeptics in obstructive lung disease was criticized on the basis of toxicity and short duration of action,[52] and the use of Tris buffer to correct respiratory acidosis was questioned on both theoretical and practical grounds.[53] Reinvestigation of the use of carbonic anhydrase inhibitors[54] and diuretics,[55] on the other hand, led two groups to suggest that indirect ventilatory stimulation through adjustment of kidney function represents a useful alternative. Although the last of these approaches is the most rational, it is obvious that none would be expected to have any effect on the underlying disorder, which must be associated with the inability of diseased lung tissue to properly carry out its function of oxygen-carbon dioxide exchange.

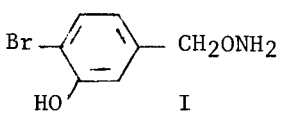
Allergy.--Glynn categorized the various types of allergic responses, reemphasizing that allergy should be considered a branch of immunology,[56] and the immunological aspects of delayed hypersensitivity were thoroughly reviewed.[57] From this viewpoint, it is axiomatic that advances in the understanding of allergic phenomena can be expected to depend on progress in the field of immunology, and that only from the latter source will emerge approaches to treatment that deal with causative factors rather than with symptoms. With the exception of corticosteroid therapy,[5,6,58] which presumably owes at least part of its effectiveness to suppression

of the immune response, most approaches continue to deal with the antagonism of inflammatory responses that probably should be considered the sequelae of some primary immunological event. Extensive reviews on the role of mast cells[59] and mediators thought to be involved in inflammatory responses[60] summarized much of the present mechanistic understanding. Buffoni reviewed the possible biological significance of histaminase[61]--an area which seems to have been relatively neglected in considering the regulation of phenomena thought to result from the action of endogenous histamine. The continued utility of antihistaminic agents for symptomatic treatment of allergic phenomena[62,63] undoubtedly assures that histamine will continue to occupy a central role in mechanistic studies in this field.

Antihistamines.--Although it is relatively easy to prepare active antihistaminic compounds with substantial elements of structural novelty, it remains difficult to identify means for introducing significant improvements. Additional clinical trials with the dibenzoxazepine derivative SQ-10648[64] and the tetrahydroquinazoline Hpt-909,[65] cited last year,[1] did not suggest impressive advantages over existing agents. The desirability of incorporating a component of antiserotonin[66] activity remains an open question.

Ash and Schild,[67] on the basis of *in vitro* studies with agonist analogs of histamine and with antihistamines, suggest that histamine stimulatory receptors in guinea pig ileum be designated $H_1$ receptors. Those responsible for stimulation of gastric acid secretion and inhibition of rat uterus represent another type which are different from $H_1$ receptors in terms of agonist structure-activity relationships and in the fact that specific antagonists are unknown for these responses. Lish and coworkers[68] provided evidence that adrenal epinephrine release contributes significantly to the protection of guinea pigs by antihistamines when the histamine challenge is given intravenously, but not when it is administered by aerosol. Accordingly, they suggest that the latter route of histamine administration should provide the more reliable estimate of antihistaminic activity. However, recent studies showing that certain antihistamines inhibit the reflex release of epinephrine in cats and dogs following histamine challenge[69] suggest that it may be extremely difficult in the whole animal to separate antihistaminic actions from adrenergic effects. These findings lead one to the conclusion that "specific" antihistamine activity is still best measured on strips of guinea pig ileum.

The alternate approach of using histidine decarboxylase inhibitors to treat disorders associated with the actions of excessive histamine was again suggested by Levine,[70] on the basis of favorable clinical results in mastocytosis with brocresine (I, NSD-1055, Cl-54998).



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New support for the validity of this idea was provided by the report that histamine biosynthesis is markedly increased following anaphylaxis in rats and guinea pigs.[71] If this phenomenon proves to be

general for other types of allergic reactions, a position long held by Schayer,[72] efficient inhibition of histidine decarboxylase may provide a means for preventing the slowly developing symptoms that have been difficult to correlate with the rapid release of histamine from mast cells.

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# Chapter 10. Agents Affecting Gastrointestinal Functions

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Secretion. In the past year, publications describing chemical substances that inhibit gastric secretion were greatly exceeded by papers devoted to the study of mechanisms of secretion induction and inhibition. Many of these were concerned mainly with gastrin and its synthetic derivatives, secretin, histamine, 2-deoxyglucose, and insulin. Although stimulation of secretion is not a major objective of medicinal chemistry, knowledge of the mode of action of stimulators and physiological conditions inducing secretion could be of value in the design of inhibitors. Therefore, appropriate papers on secretion stimulation and mechanism have been included in the present review.

Gastrin and Derivatives. Recovery of gastrin activity from antral mucosa by the use of techniques such as ultracentrifugation, ultrafiltration, and Sephadex gel filtration to minimize fragmentation of proteinaceous material has indicated to one group of investigators[1] that gastrin, as it exists in the antral mucosa, is a larger protein than the heptadecapeptide isolated by Gregory and Tracy.[2]

A synthetic derivative of an active fraction of gastrin, commonly referred to as the "gastrin-like pentapeptide" or ICI 50,123, has been further evaluated in animals and humans and has been used as an investigational secretory stimulant. This pentapeptide (I) consists of the tetrapeptide unit, TryMetAspPheNH$_2$, from the carboxyl end of the gastrin molecule, acylated on the tryptophan amino group with N-(tert-butyloxycarbonyl)-β-alanine.[3]

$$(CH_3)_3COCO\text{-}\beta\text{-}AlaTryMetAspPheNH_2 \qquad I$$

ICI 50,123 increased secretion in the perfused rat stomach where it had a more rapid onset and shorter duration of action than gastrin but was only about one-eleventh as potent.[4] It was similar to gastrin in that large doses caused an inhibition of secretion in dogs.[5]

In the human, ICI 50,123, administered parenterally, caused practically no circulatory or blood pressure changes.[6] Peak stimulation of gastric output was obtained by i.v. infusion of 0.01-0.1 μg./kg./min.[7] In a single-dose evaluation, 6 μg./kg. of ICI 50,123 was equivalent to 40 μg./kg. of histamine acid phosphate.[8] No synthetic derivatives of gastrin, other than those already reviewed,[9] have been described.

The maximum stimulatory response of acid output elicited by gastrin in man was higher than the maximum response to histamine or histalog.

At lower doses, a synergistic effect between histamine and gastrin was found but the maximum secretory effect of gastrin alone was not potentiated. Atropine had a distinct inhibitory effect on the maximum secretory response to gastrin.[10] The patterns of electrolyte and acid secretion resulting from gastrin stimulation in man were found to be very similar to those following histamine stimulation.[11] Secretion of intrinsic factor in man was stimulated by gastrin II.[12]

In the denervated Heidenhain pouch of the oxyntic gland area in dogs, the maximum acid secretory response to gastrin II and secretion stimulating peptide (ICI 50,123) was lower than to histamine.[13] Gastrin has been shown to have a stimulating effect on acid secretion from the isolated bullfrog mucosa.[14]

Insulin and 2-Deoxyglucose. The stimulation of gastric secretion by insulin and 2-deoxyglucose and the mechanism of their action has received increased attention.

In pylorus-ligated rats, 2-deoxyglucose (2DG) (600 mg./kg.) administered subcutaneously evoked maximum stimulation.[15] By the same route of administration in chronic fistula rats, the gastric response was all or none and the threshold dose was approximately 50 mg./kg. Atropine abolished the gastric response to 2DG in these rats.[16] The stimulatory effect of 2DG on dogs with gastric fistulas was completely eliminated by vagotomy. However, 2DG and insulin both stimulated secretion in a Heidenhain (denervated) pouch in dogs also having an open gastric fistula (innervated). Closure of the fistula allowed acid from the stimulated stomach to bathe the antrum and duodenum resulting in inhibition of the Heidenhain pouch secretion. The authors suggest that these data demonstrate vagal mediated release of gastrin from the pyloric gland area by 2DG and insulin.[17] In similar type experiments, other investigators suggest that inhibition of secretion in a Heidenhain pouch by insulin may be due to liberation of glucagon.[18]

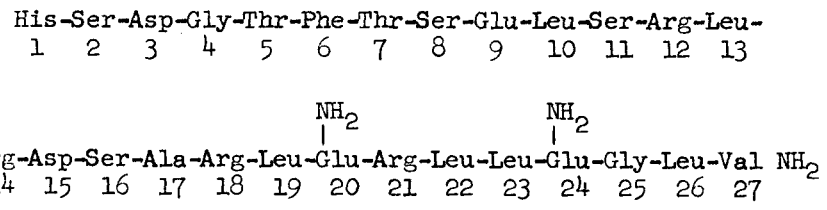
Rats made diabetic by administration of alloxan showed an inhibition of acid secretion which was suggested to be purely the result of the hyperglycemia following the destruction of the beta cells of the pancreas.[19] However, hyperglycemia induced by parenteral administration of glucose, did not inhibit histamine stimulated gastric secretion in rats.[20] In human diabetics a decrease in gastric secretory function was associated with elevated fasting blood glucose levels.[21]

After a normal stimulatory effect, high doses of insulin ($>0.45$ U/kg.) in dogs with gastric fistula showed a consistent inhibitory effect on histamine-stimulated acid secretion[22] with the earliest observed effect being a reduction of $K^+$ in the gastric juice.[23] Restoration of the blood sugar levels by i.v. administration of glucose did not reverse the inhibition of acid and $K^+$ output, showing that the inhibition was not related directly to the insulin hypoglycemia.[24] Administration (i.v.) of potassium chloride (1 milliequivalent/kg.) completely and rapidly reversed the insulin inhibition of gastric electrolyte excretion. These results

indicate that the insulin inhibition might be the result of a redistribution of intracellular $K^+$ with depletion of $K^+$ from sites essential to secretion.[25] Potassium chloride also modified the inhibitory effect of insulin in nonhistamine-stimulated animals.[26] Rats on a potassium deficient diet for four weeks also showed a diminution of gastric acid secretion which was accentuated by administration of excess mineralocorticoid.[27]

Secretin, an intestinal hormone obtainable from the duodenum, particularly on acidification, has been used clinically as a diagnostic aid in pancreatic disease to stimulate pancreatic secretion. It has been well-established that secretin also inhibits gastric acid secretion. The most recent investigation showed that secretin inhibited gastrin-stimulated secretion but not that induced by histamine in dogs with a Heidenhain pouch.[28] Insulin release by the pancreas is stimulated by secretin in dogs[29] and in man.[30]

The preparation of very pure secretin[31] has been described and degradation studies[32,33] on the pure hormone have permitted a structural assignment to be made.[34,35] The assigned structure is that of a straight chain polypeptide consisting of twenty-seven amino acid units (II). It shows a resemblance to glucagon in that 14 of the amino acid units (No. 1, 2, 4, 5, 6, 7, 8, 11, 15, 16, 18, 20, 24, and 26 in II) in secretin occupy the same position in the peptide chain as they do in glucagon.

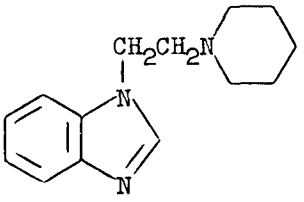



II

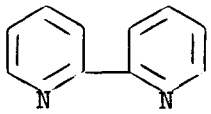
The structure proposed for secretin was synthesized by Bodanszky and coworkers[36] by two different methods. In one, the heptacosapeptide chain was formed by stepwise addition of individual amino acid units and in the other, four peptide fragments were combined. However, none of the synthetic preparations have been as potent as the purified secretin from natural sources.

Inhibitors of Gastric Secretion. Various other hormonal polypeptides have been the subjects for gastric secretory investigation. Bradykinin showed an inhibitory effect on gastric acid secretion in dogs.[37] Angiotensin caused a modest inhibition of food induced secretion volume in Pavlov pouch dogs.[38] Secretion volume but not acid concentration was reduced in dogs with a Heidenhain pouch by antidiuretic hormone. Oxytocin was without effect.[39]

A series of 1-(dialkylaminoalkyl)benzimidazoles having an inhibitory effect on gastric secretion has been reported.[40] One representative, 1-(2-piperidinoethyl)benzimidazole (III) had an $ED_{50}$ of 11.2 mg./kg. for total acid in the rat. At 50-100 mg./kg. p.o. gastric emptying time was increased and intestinal motility was inhibited. It was concluded that parasympathetic transmission was selectively blocked.



III



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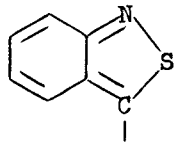


V

The effect of four adrenergic blocking agents on gastric secretion in dogs has been reported by Pradhan and Wingate.[41] Phentolamine and tolazoline induced secretion at 1.2 mg./kg. i.p. Phenoxybenzamine at 20 mg./kg. i.p. and dichloroisoproterenol at 10 mg./kg. i.p. did not induce secretion and both blocked bethanechol induced stimulation in some experiments and histamine induced stimulation (volume and acidity) in all experiments. Under certain conditions, the secretion inhibiting effect of adrenergic agents (isoproterenol and epinephrine) was partially blocked by dichloroisoproterenol leading to the conclusion that adrenergic inhibition of gastric secretion is mainly a β-receptor function.

2,2'-Bipyridine (IV) decreased volume, hydrogen ion, and pepsin output in pylorus-ligated rats but had no effect on insulin- or histamine-stimulated secretion in dogs.[42]

3-Methylamino-2,1-benzisothiazole (V) (7.2 mg./kg.; s.c. and p.o.) caused a sharp reduction in volume and approximately 10% reduction of hydrogen ion concentration in pylorus-ligated rats. A dose of 20 mg./kg. i.v. in histamine- or insulin-stimulated dogs with innervated gastric pouches resulted in volume reductions up to approximately 50% and a very small but statistically significant decrease in acid concentration.[43]

The effects of p-chloromercuribenzoic acid, N-ethylmaleimide and iodoacetamide on various enzymes in the gastric mucosa were studied by histochemical methods. These compounds block gastric acid secretion and also inhibit sulfhydryl-containing enzymes. No conclusion was reached as to whether these inhibitors act by blocking energy-producing systems or by inhibiting a specific enzyme system involved in the production of hydrogen ions.[44]

The action of adenosine-3',5'-monophosphate (6-8 mg./kg., i.v.) on the human gastrointestinal tract has been determined. Intestinal motility was promptly abolished and, after histamine stimulation, acid output was depressed 35±17% although no significant change in volume or pH was

reported.[45]

Opipramol (5-[γ-(β-hydroxyethylpiperazino)propyl]-5H-dibenzo(b,f)-azepin dihydrochloride) reduced gastric hyperacidity in humans.[46]

The inhibitory effect of various anticholinergic agents on gastric secretion in the human has been the subject of several publications. These agents include glycopyrrolate,[47] poldine,[48] tropenzilene[49] (benzilic acid ester of 7-methoxy-N-methyltropinium bromide), and piribenzil[50] (benzilic acid ester of N,N-dimethyl-2-(hydroxymethyl)piperidinium methyl sulfate). Three quaternary ammonium salts of the general structure benzyl tri(β-alkoxyethyl)ammonium iodide had an inhibitory effect on gastric secretion in Shay rats. These compounds were reported to be non-anticholinergic.[51]

In humans, estrogens had no effect on the secretion of acid or pepsin and probably increased the production of mucus in the stomach. However, they appeared to control the symptoms of duodenal ulcers.[52]

In human ulcer patients, heparin did not significantly reduce basal gastric acid secretion but produced a significant inhibition of the response to histamine and insulin stimulation.[53] In guinea pigs, heparin also markedly inhibited the gastric secretory response to histamine but in betazole (3-(2-aminoethyl)pyrazole) stimulated animals there was an increase in secretion volume.[54] It was concluded that heparin may have its effect by binding with histamine. Heparin significantly inhibited secretion in dogs stimulated with food, insulin, acetylcholine, methacholine, 48/80, histamine, and gastrin.[55]

Ulcers. The relationship between acid and pepsin,[56] nutritional state,[57,58] histamine[59] and histidine[60] metabolism, sulfate synthesis and $S^{35}$ uptake,[61] hypophysectomy,[62] sex hormones,[63] and other factors[64,65] on the production of "stress" or drug induced ulcers has been studied.

Amitriptyline (2.5-10 mg./kg. s.c.) protected male rats against immobilization ulcers, while chlordiazepoxide (5-50 mg./kg.) had no protective effect.[66] When given concurrently, however, the protective effect was 1.7 fold greater with the combination than with amitriptyline alone. Nortriptyline, chlorpromazine, and imipramine all were found to prevent Shay ulcers in rats.[67] These agents also increased mucin content in the gastric juice. Several pyrimidines (4-methyluracil, 4,6-dihydroxypyrimidine, 2-methyl-4,6-dihydroxypyrimidine, cytosine) caused a 42-72% reduction in gastric ulceration produced by repeated oral administration of caffeine.[68] These compounds apparently stimulate regenerative processes and complement the effect of other therapeutics in the treatment of gastric ulcers.

Halidor (1-benzyl-1-(3-dimethylaminopropoxy)cycloheptane fumarate) reduced the ulcer formation due to 16-20 days of glucose feeding in rats.[69] Restraint and serotonin ulcers were also blocked. Phenindione (2-phenyl-1,3-indandione) therapy in man was reported to have induced hemorrhagic ulcerative colitis.[70]

Powdered aspirin, placed on dog explanted mucosa, blocked histamine-stimulated acid secretion. Edema and hemorrhagic petechiae were observed on the explant.[71] Aspirin ingestion in dogs caused bleeding from isolated gastric pouches which was dose-related.[72] Choline salicylate did not produce these changes. Aspirin in suspension or in either of two precipitated solutions at pH 3.0 produced gastric hemorrhage in Pavlov pouch dogs. Salicylic acid, methyl salicylate, salicylamide or acetaminophen solutions did not produce hemorrhage. Neither of the two groups of agents produced hemorrhage when given at pH 6.5.[72a]

Carbonic anhydrase inhibitors decreased the incidence of phenylbutazone induced ulcers in rats with acetazolamide giving complete protection. There was good correlation between diuretic activity of the carbonic anhydrase inhibitors and the degree of antagonism of the ulcers. This was cited as support for the contention that the ulcerogenic activity of phenylbutazone is partly due to stimulation of carbonic anhydrase in the gastric mucosa.[73]

Motility. Several new methods for the study of gastrointestinal motility in unanesthetized dogs were published in the last year. All utilized chronically implanted transducers attached to the serosal surface of the stomach or intestine. Reinke and coworkers used modified extraluminal strain gage transducers to record contractile activity and tone from circular and longitudinal muscle of stomach, small intestine, and colon in the unanesthetized dog.[74] The effect of 5-hydroxytryptophan, neostigmine, morphine, histamine, and hexamethonium on the patterns of contractile activity were described. Rosenbaum et al. analyzed parameters such as shape, force, frequency, and velocity of circular and longitudinal muscle contractile activity.[75] Nelson et al. studied the effect of insulin and feeding on motor activity of the circular muscle of the stomach.[76] Renecker and Brendel measured rhythm and intervals of "peristalsis" in the stomach, small intestine, and colon.[77] Atropine, neostigmine, and cholecystokinin were studied, none of which altered the frequency of contraction. Passage of a charcoal meal in mice was facilitated by neostigmine, pilocarpine, barium chloride, 5-hydroxytryptophan, and castor oil and inhibited by anticholinergics, papaverine, and ganglionic blockers.[78]

In a study of acetylcholine receptors in longitudinal muscle of guinea pig small intestine, using tritium-labelled atropine, three components could be distinguished:[79] (1) a binding site with a capacity of 180 picamoles/gm., (2) a binding site with capacity about 1,000 picamoles/gm., and (3) a nonsaturable compartment with a clearance of 4.7 ml/gm. Woolley and Gommi showed that serotonin receptors in an isolated strip of rat stomach could be specifically destroyed by two treatments with purified neuraminidase plus ethylenediaminetetracetic acid.[80] The response to serotonin could be recovered by the addition of purified gangliosides indicating these make up part of the receptor.

An evaluation of drug effects on motility of isolated strips of human gastrointestinal tract was made.[81-83] The pylorus was found to resemble other areas of the gut in its response to drugs but the cardiac

and ileocecal sphincters were pharmacologically distinct, being contracted by epinephrine and acetylcholine. Cholinergic agents, norepinephrine, and epinephrine caused contraction of cat longitudinal esophageal smooth muscle. The action of the latter two compounds was blocked by tolazoline and atropine.[84] Isoproterenol inhibited esophageal smooth muscle and was antagonized by propranolol. The effects of neurohumors and drugs on the electrical and contractile responses of the pyloric region were reported by Daniel.[85,86] Adrenergic agents, cholinergic agents, histamine, serotonin, phenylbiguanide, and morphine were all studied and their mechanism and site of action analyzed.

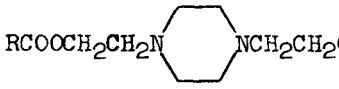
Commercial preparations of cholecystokinin stimulated peristalsis in the colon of man[87] and gall bladder, stomach, and small intestine in the dog.[88] In dogs, secretion of natural cholecystokinin stimulated by jejunal perfusion with 0.1 N HCl did not produce an increase in small intestinal motility but did produce gall bladder contraction.[88]

Gastrin pentapeptide (I) in doses producing near maximal secretion of gastric acid (0.01 mg./kg./min.) increased motor activity of the antrum in man.[89] Higher doses of pentapeptide were needed to stimulate colonic activity in man.[90] Gastrin II in doses of 25 or 50 μg. i.v. was found to increase motor action of the distal colon and rectum, however, the effect was more pronounced in the proximal portion of the alimentary tract.[91] Doses used were 10 times the amount necessary to elicit acid secretion.

Glucagon (9-180 γ/kg.) depressed neostigmine induced motility of stomach, duodenum, and colon.[92] No correlation between duration of inhibition and arterio-venous glucose differences was noted.

In a study of the effect of various narcotic analgesics on the pyloric sphincter in rats, morphine (0.5 mg./kg.), meperidine, and papaverine caused an increase in flow through the pylorus, but dextromoramide and diphenoxylate (ethyl 1-(3-cyano-3,3-diphenylpropyl)-4-phenylisonipecotate) decreased flow.[93]

Several different types of antispasmodics have been described. Most of the agents are anticholinergics, however a few appear to have other activities. Nafiverine (VI, R = 1-(α-naphthyl)ethyl) showed anticholinergic, antihistaminic, and antiserotonin activity and blockade of $BaCl_2$ spasm on isolated ileal muscle.[94] The effect on activity of variations of R in VI have been reported.[95] Piribenzyl (see ref. 50) inhibited spasm induced by acetylcholine, histamine, and $BaCl_2$ and inhibited intestinal motility in dogs in doses of 0.02-0.05 mg./kg. i.v.[96] In man this compound inhibited gastric emptying and small intestinal transit.[97] N-Methylbis-(4-chlorophenoxy)acetamide showed activity against acetylcholine, and $BaCl_2$ spasm of isolated rat duodenum and was effective in



VI

inhibiting intestinal motility in the mouse.[98] It also blocked histamine induced contraction of guinea-pig ileum, histamine hypotension in dogs, and serotonin diarrhea. Nicotinyl dihydrocodeine in doses of 8 mg./kg. i.v. decreased peristalsis and tone of dog duodenum as recorded by the balloon technic. Barium chloride and neostigmine stimulation were inhibited.[99] The results are the reverse of those obtained with dihydrocodeine.

Clinical studies indicate that malethamer, a crosslinked copolymer of ethylene and maleic anhydride, was effective in the treatment of diarrhea due to its unusual water binding capacity.[100,101] Anisotropine methyl bromide (2-propylpentanoyltropinium methyl bromide) and phenobarbital provided greater relief for various functional gastrointestinal disorders than the anisotropine alone or belladonna alkaloids with phenobarbital.[102]

Ulcerative Colitis. The use of immunosuppressive drugs (6-mercaptopurine, busulfan, 6-thioguanosine, azathioprene) in experimental therapy of ulcerative colitis has been reported.[103-106] Results have been encouraging in the small group of patients tested but potentially serious side effects were observed due to the high doses that were required. Disappointing features are the failure to achieve a cure and the severity of relapse on suspension of treatment.

Antiemetics. Diphenidol (1,1-diphenyl-4-piperidinobutanol) has been reported to have antiemetic activity equal to chlorpromazine, but without many of the central nervous system actions of the phenothiazines.[107] It blocked apomorphine induced emesis by oral, i.m., and rectal administration. In clinical trials dephenidol was found to be more effective than a placebo for relief of nausea and vomiting, with few side effects.[108,109] It was also found to be effective against various types of vomiting in man in an uncontrolled experiment.

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## Section III - Chemotherapeutic Agents
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### Chapter 11. Antibiotics and Related Compounds
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General. -- References cited in this brief report are intended as guides to the most notable chemotherapeutic advances in this vast field during 1966. Antiviral, antifungal and antineoplastic antibiotics are found in Chapters 13, 15 and 16 respectively.

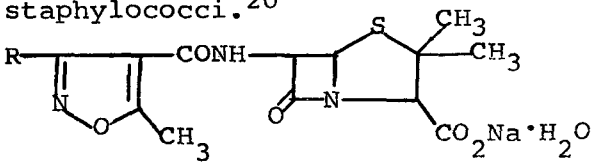
The alarming increase in serious infections caused by drug-resistant gram-negative bacilli[1,2] is attributed in part to the ominous phenomenon of "infectious drug resistance" in enteric bacteria[3,4] (cf. Chapter 12) discovered in Japan in 1959.[3] The majority of all antibiotic-resistant strains of Escherichia coli, Aerobacter, Proteus, Pseudomonas, Klebsiella, and Salmonella from clinical sources are capable of transferring multiple-drug resistance (R factors) to sensitive strains of enteric bacteria in mixed culture.[5,6,7] The in vivo transfer of drug resistance has been demonstrated.[8] Genes responsible for the resistance can be carried on extrachromosomal DNA. A deeper insight into the biochemical and genetic bases of drug resistance[9] may hopefully lead to more effective countermeasures against this "public-health hazard".[7]

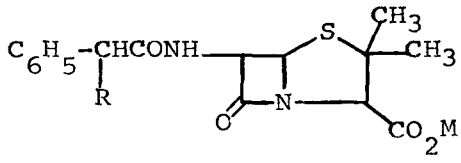
The β-lactam antibiotics continued to be the cynosure of synthesis. Some structure-activity relationships of penicillins[10] and cephalosporins[11,12] were discussed. Timely reports aimed primarily toward the evaluation of the newer highly serum-bound penicillins dealt with the controversial relationship of drug-serum protein binding to clinical antimicrobial effectiveness.[13,14]

The crowning intellectual achievement of the year was Woodward's[15] brilliant total synthesis of cephalothin (X) and cephalosporin C (XIII) via a key intermediate presumably useful for the synthesis of unique variants of both cephalosporins and penicillins.

Uses of chemically defined media for producing antibiotics were reviewed.[16] An excellent book on the biosynthesis of antibiotics was published.[17] A practical book for physicians and clinical laboratory personnel became available.[18]

Penicillins. -- Dicloxacillin (III) was effective in 48 of 49 patients afflicted with pneumococcal and staphylococcal wound infections.[19] Against clinical isolates of S. aureus and S. epidermidis, dicloxacillin was 2 to 3.5 times more active than cloxacillin (II) and oxacillin (I).[19] Other clinicians found dicloxacillin highly efficacious for oral treatment of infections caused by streptococci and penicillinase-producing staphylococci.[20]





I R = phenyl

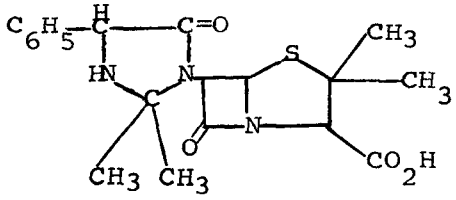
II R = 2-chlorophenyl

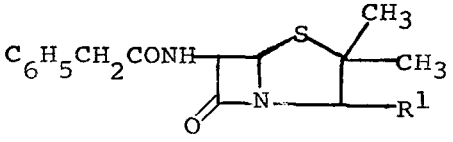
III R = 2,6-dichlorophenyl

IV R = $NH_2$; M = H

V R = H; M = K

Ampicillin (IV), by virtue of its broad spectrum, continued to be of interest for the treatment of various infections caused by gram-negative organisms. In a comparative study, ampicillin was active against 43% of 109 strains of E. coli whereas benzylpenicillin (V) was active against only 9.9% of the same group.[21] Synergism between ampicillin and several β-lactamase-resistant penicillins was observed in the laboratory[22,23,24] and also in a clinical study involving urinary-tract infections.[25]





VI

VII $R^1 = CO_2CH_2OCOCH_3$

VIII $R^1 = CHO$

Hetacillin (VI)[26] has given higher human serum concentrations than those obtained with ampicillin.[27] Twelve groups of clinicians studied the effect of hetacillin in over

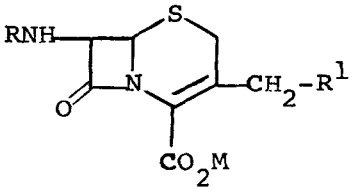
865 patients afflicted with a variety of gram-positive and gram-negative infections. Notably satisfactory clinical results were reported.[28]

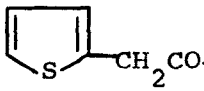
After oral administration to the dog, penamecillin (VII), which is the acetoxymethyl ester of benzylpencillin, produces prolonged benzylpencillin serum levels.[29] According to pharmacokinetic evidence[30], the penamecillin is slowly absorbed unchanged throughout the intestinal tract and then is rapidly hydrolyzed to benzylpenicillin by nonspecific esterases. Compound VIII is an example of the hitherto unknown penicillin aldehydes for which a general synthesis was developed.[31]

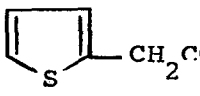
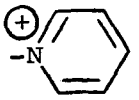
Further observations were published concerning the mechanism of action of the penicillins.[32-35] Data support the concept that the β-lactam antibiotics inhibit a transpeptidase essential for a cross-linking reaction in bacterial cell-wall synthesis. Transpeptidase derived from cell-free systems of E. coli, like that from S. aureus, is inactivated by penicillins. Thus, the possession of cellular permeability barriers in addition to β-lactamase productive capacity apparently tends to protect gram-negative bacteria from lethal attack by β-lactam antibiotics.[33]

A reliable and rapid procedure was described[36] for the iodometric detection of staphylococcal penicillinase in clinical microbiology.

Cephalosporins. -- Many patients with a history of penicillin hypersensitivity have shown no allergic response to cephalothin (X)[37,38,39] or cephaloridine (XI).[40,41]



X R =  $CH_2CO-$; $R^1 = -OCOCH_3$; M = Na

XI R =  $CH_2CO-$; $R^1 =$  ; M = ⊖

XII R = D-$C_6H_5CH(NH_2)CO-$; $R^1 = -OCOCH_3$; M = H

XIII R = D-$HOOC-CH(NH_2)(CH_2)_3CO-$; $R^1 = -OCOCH_3$; M = H

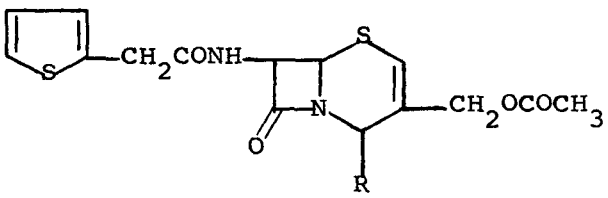
XIV R = 2,6-$Cl_2C_6H_4CO$-; $R^1$ = $-OCOCH_3$

In laboratory tests cephaloridine was generally more active than cephalothin against gram-negative organisms.[40,42] In man, average peak serum levels of cephaloridine were double those of cephalothin and more prolonged.[40,42,43] Urinary excretion of cephaloridine was about twice that of cephalothin.[40] Anemia developed in several patients receiving cephaloridine.[41] Some toxic reactions associated with cephaloridine therapy were attributed to the low renal clearance of the drug combined with its notable stability in the body.[43] Cephaloridine, however, was less effective against penicillinase-producing staphylococci than cephalothin.[42] An improved synthesis[44] of cephaloridine was utilized to prepare a series of analogs.[12]

A clinical trial[45] established cephaloglycin (XII)[46] as the first reported orally effective cephalosporin. Cephaloglycin, cephalothin and cephaloridine showed comparable activity against most of the gram-negative pathogens tested.[45] The known instability of cephaloglycin in solution[46] may have contributed to the disturbing side effects noted, namely gastrointestinal distress and severe diarrhea.[45]

Semisynthetic cephalosporins, such as XIV were found to protect cephaloridine and cephalothin from decomposition by the enzymes of certain gram-negative bacteria. Because mice can be protected from hitherto resistant infections with a mixture of the enzyme inhibitor and cephaloridine, for example, such combinations may become therapeutically important.[48]

Most methods used for the preparation of amides and esters of cephalothin (X) gave rise to the isomeric $\Delta^2$-cephalothin derivatives (XV) which have decreased antimicrobial activity.[47]




XV

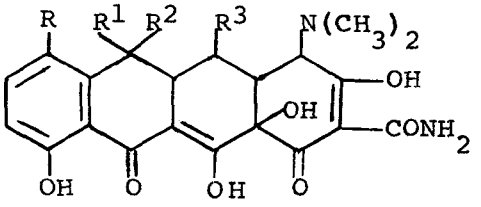
R = amide or ester moiety

It is known that dry pyridine and other organic bases can appreciably isomerize the $\Delta^2$-compounds to the corresponding cephalosporin derivatives.[15,49]

Recent publications[50,51,52] have presented substantial evidence for possible cross-allergenicity of the penicillins and cephalosporins. However, direct interaction of the β-

lactam of the penicillin nucleus with an amino group of carrier protein (penicilloylation) seems to be the most important mechanism for antigen formation in penicillin allergy.[53] Striking differences between the known chemical behavior of a penicillin β-lactam and a cephalosporin β-lactam ring system, even when the side chains are identical, suggest statistically significant differences in sensitizing potential. Furthermore, the nature of the side chain in a penicillin is an important factor in determining antigenic liability.[52]

Tetracyclines. -- Methacycline (XVI), marketed in the U.S. in September, was recently evaluated in the treatment of respiratory-tract infections.[54]



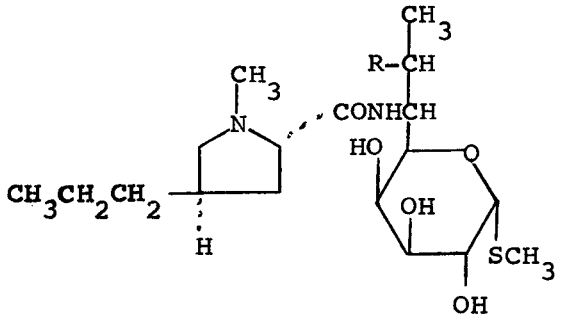

XVI R = H; $R^1R^2 = CH_2$; $R^3$=OH

XVII R = H; $R^1 = CH_3$; $R^2$ = H; $R^3$ = OH

XVIII R = $N(CH_3)_2$; $R^1$ = H; $R^2$ = H; $R^3$ = H

Doxycycline (XVII), also a chemical modification of oxytetracycline, gave high, protracted blood levels and showed an activity comparable to demethylchlortetracycline.[55] Minocycline (XVIII)[56,57] was found more potent than any other reported tetracycline. Efficient oral absorption in animals, unique effectiveness against tetracycline-resistant strains of staphylococci and useful activity against M. tuberculosis H37Rv are all properties attributed to minocycline.

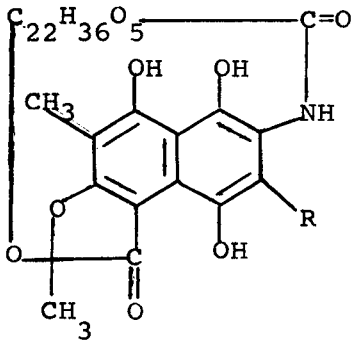
Lincomycin. -- Antimicrobial potency and spectrum of lincomycin (XIX) have been modified significantly by structural alterations involving changes in the two substituents on the pyrrolidine ring and by replacement of the 7-hydroxy group with a chlorine or a bromine atom.[58] For example, changing the $N-CH_3$ to $N-C_2H_5$ preserved the gram-positive potency and enhanced the gram-negative activity about tenfold. 7-Chloro-7-deoxylincomycin (XX) proved several times more active than lincomycin in vivo against gram-positive bacteria but was inactive, as is the parent, against gram-negative bacteria. Atleast ten U.S. patents concerning various lincomycin derivatives were issued during 1966. Further analogs are under investigation.



XIX R = OH

XX R = Cl

Rifamycins. -- Systematic chemical modification of rifamycin SV (XXI) has led to remarkable chemotherapeutic improvements which include broadening the antimicrobial spectrum to include gram-negative bacteria, gaining oral activity and obtaining higher stability. Such a derivative is exemplified by rifaldazine (XXII)[59,60] which has shown clinical promise in the treatment of respiratory and urinary-tract infections, osteomyelitis and tuberculosis.



XXI R = H

XXII R = CH=N-N N-CH$_3$

XXIII R = $CH_2N(CH_3)_2$

Mannich bases (XXIII)[61] of rifamycin SV and rifazine,[62] a phenazine derivative of rifamycin SV, are other examples of enhanced therapeutic efficacy through structural changes in the natural product.

Gentamicin. -- This 2-deoxystreptamine-containing amino-glycosidic antibiotic is related to the neomycins, the paromomycins and kanamycin. Clinical reports indicated gentamicin is a drug of choice for parenteral and topical treatment of infections caused by Pseudomonas aeruginosa[63,64] and Proteus mirabilis.[65] Forty-seven of 50 patients topically treated for various bacterial infections were cleared of

infection. No irritation or sensitization was caused by the drug.[66]

Kasugamycin. -- The unique structure of kasugamycin was established as an aminoglycoside containing a carboxyamidino grouping.[67] This inhibitor of polypeptide synthesis[68] has low toxicity and it is of particular interest for treatment of Pseudomonas infections.

Spiramycin. -- Hydrolytic removal of the mycarose sugar component of this macrolide antibiotic produced neospiramycin which is twice as active as spiramycin against S. albus and E. coli.[69] Because of facile deacetylation in vivo acetylspiramycin failed to show any clinical advantages over the parent spiramycin.[70]

Kanamycin. -- With the exception of Pseudomonas infections, kanamycin has proved to be a valuable antibiotic for the treatment of drug-resistant tuberculosis and serious infections caused by gram-negative bacilli.[71]

Capreomycin. -- Because of low toxicity and remarkable activity against drug-resistant strains of M. tuberculosis, capreomycin, a polypeptide antibiotic, was recommended for use in multiple-drug therapy.[72]

Chloramphenicol Analogs. -- Microbial kinetics have permitted a more precise quantification of substituent effect on antimicrobial activity in a series of chloramphenicol analogs.[73]

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# Chapter 12. Synthetic Antibacterial Agents

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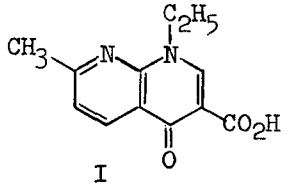
Perspective.—Work on these agents in 1966 was directed toward improvement in the level of activity or in the pharmacology of known active structures. No new antibacterial type active in animals or in man was reported.

Several sulfanilamides and other synthetic antibacterials as well as the antibiotics are involved with "infectious drug resistance."[1,2,3] Infectious drug resistance denotes the transmission of drug resistance from one bacterium (so far only Gram-negatives) to another of the same or different strain, species, or genus. This is accomplished by conjugation: formation of a temporary intercellular connection between two sex types and unidirectional transfer of the resistance genes and a transferability factor, usually associated with an extra-chromosomal sex-determining factor.[4] Conjugation leads directly to a new recipient strain with a comparable level of resistance. The surprising facet of this phenomenon is the simultaneous transfer of resistance to a whole series of chemically and biochemically unrelated antimicrobials (most commonly sulfanilamides, tetracyclines, streptomycin, and chloramphenicol but also more recently penicillins, kanamycin, neomycin, nalidixic acid and nitrofurans). There appears to be a close genetic relationship between the resistance genes which leads to their becoming arranged immediately adjacent to one another in the genetic element (DNA) transferred.[5,6] In spite of requiring several minutes and the proper physiological conditions, conjugation does occur in vivo, even though less readily[1,7] than in vitro. Resistant bacteria can be "cured" of infectious drug resistance with various acridines in vitro, albeit with low frequency;[1,2,3,5] usually the cure applies to all the resistance factors but some segregation has been achieved.[5,6]

The true chemotherapeutic significance of this phenomenon is not defined at the moment[8] but it requires careful scrutiny since it involves so many drugs and so many organisms (Coli, Salmonella, Klebsiella, Shigella, Vibrio). Perhaps, by means of this new look at a bacterially old process, we will now be able to devise a cure for this "infectious disease" of bacteria which will be effective in vivo and will finally enable us to deal with the important problem of bacterial resistance (cf. a clinical study[9] of atebrin in combination therapy of multi-drug-resistant urinary infections).

In Vivo Actives.—The Gram-negative activity of nalidixic acid I is seen from further work to be very structurally specific. Several 1,5-naphthyridine analogs apparently had little, if any, activity in vitro or in vivo while one 1,6-analog (3-carboxy-4-oxo-1,5,7-trimethyl-1,6-naphthyridine) was partially effective against Kleb. pneumoniae in mice (70% survival with subcut. 200mg/kg/day).[10] The intermediate diethyl N-(2,6-dimethyl-4-pyridyl)amino-methylenemalonate had some in vivo Staph. activity (60% survival

with subcut. 100mg/kg/day). Curiously, three 1,7-naphthyridines[11] had only Gram-positive activity: 1,6,8-trimethyl-3-carboxy-4-oxo-1,7-naphthyridine was active against Staph., Strep. and Pneumococcus (partly effective) at 25, 100 and 400mg/kg/day orally in mice; the 1,8-dimethyl-6-ethyl and the 1-ethyl analogs were much less active.

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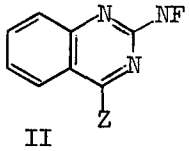


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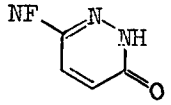


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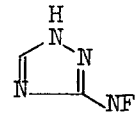
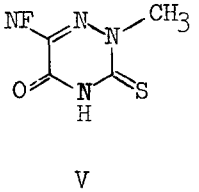



IV

Several types of nitrofurans (NF = 5-nitro-2-furyl) with interesting levels of in vivo activity were reported but margins of safety were not given. The 4-aminoquinazolines II [Z = $N(CH_3)C_2H_4OH$, $N(C_2H_4OH)_2$ and $NHC_4H_8$-1-pyrrolidinyl] had a broad in vitro spectrum, including Pseudomonas, Proteus and Aerobacter, and high activity (0.01-12mcg/ml).[12] However, only activity in vivo against Staph. aureus was reported ($ED_{50}$ oral 30-80, intraperitoneal 1-15mg/kg); activity correlated poorly with the in vitro data. Homologs of $CH_3$ were less active. Pyridazinone III and its dihydro intermediate[13] were about equal in vivo and in vitro in Salm.-Staph. activity; 2-alkylation lowered the in vitro activity of both. In contrast, it increased the in vivo activity of the former and decreased that of the latter. Their in vitro activity (min. inhib. concn. 2-6mcg/ml) was less than that of II (0.05-0.8mcg/ml) while the in vivo activity was the same. Also poorer in activity in vitro and in spectrum was the triazole IV which had comparable Salm.-Staph. activity in vivo ($ED_{50}$ 20-40mg/kg).[14] 1-Acylation or 5-alkylation decreased its activity which was greater than that of the open-chain N-formylamino nitrofuramidine, in turn more active in vivo than the amidine itself. The 1-methylcarbamoyl-5-imino derivative of IV was active[15] against Staph., Salm. and Coli in mice at about 40mg/kg with an oral $LD_{50}$ of 1900. A series of 2-(5'-nitro-2'-furyl)CH=CH— pyridines showed the following single intraperitoneal anti-Salmonella activity ($ED_{50}$ 2-10mg/kg; oral $ED_{50}$ 5-75mg/kg) relationship in mice: 6-$CH_2OH$-1-oxide = 5-$CH_2OAc$-1-oxide > 6-$CH_2OAc$-1-oxide > 5-$CH_2OAc$ > 6-$CH_2OH$ = 5-$CH_2OH$ > 6-$CH_2OAc$ with approximately the same relationship of toxicity.[16] The 5-methyl-1-oxide reported last year had comparable activity. The as-triazine derivative V was partly effective[17] against Staph. and Salm. infections in mice at 100mg/kg. Activity of 5-methylmercaptomethyl-3-(5'-nitrofurfuryl-ideneamino)-oxazolidin-2-one was unpromising[18] in various urinary infections in a small clinical trial.

Further clinical investigations have confirmed the efficacy of ethambutol (EMB), (+)-N,N'-bis(1-hydroxy-2-butyl)ethylenediamine, in treatment of pulmonary tuberculosis, and also demonstrated its use against renal and vesical tuberculosis,[19] and in the treatment of pulmonary infections due to atypical mycobacteria.[20,21,22] In controlled trials conducted in the National Sanatoria of Japan[23] to determine the best third component of a triple regimen with isoniazid (INH) and streptomycin (SM) in original treatment, cycloserine and p-amino-salicylic acid (PAS) were less effective and more toxic than EMB while ethionamide was as effective as EMB but less well tolerated (cf. a dose-response comparison in monkeys[24]). In a

companion trial,[25] ethambutol (1g daily) combined with either cycloserine (0.5g daily) or ethionamide (0.5g daily) was well tolerated and highly effective in tuberculosis re-treatment (resistant to SM, INH and PAS). In these trials a diminution in visual acuity occurred in about 2% of the patients receiving 1g EMB daily; the effect was reversible. This incidence and reversibility was observed in other clinical studies.[26,27,28,29] A reduced dosage regimen[30] avoided the visual disturbance. While EMB by itself is effective against acute and chronic tuberculosis,[28,30,31] combined therapy is recommended to avoid emergence of resistant mycobacteria. In man, about 10% of EMB is metabolized[27] to the inactive dicarboxylic acid by the sequence $-CH_2OH \rightarrow -CHO \rightarrow -CO_2H$. A structure-activity study[32] has defined the very specific requirements for activity in an extensive series of EMB analogs.



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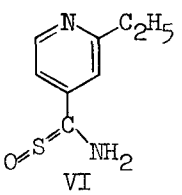

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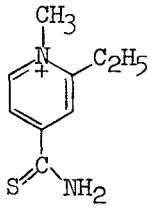

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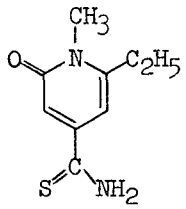


VIII

In combination with SM, ethionamide (ETH, 1g daily) was as effective as INH (0.3g daily) in man by three therapeutic criteria. However, the ETH regimen was considered to be too toxic to be clinically acceptable.[33] Clinical incidence of side-effects from ETH (plus INH) decreased sharply with dosage: 31% for 1g, 18% for 0.75g and 8% for 0.5g daily.[34] Separation and characterization of ETH metabolites has been achieved and their quantitative interrelation is under study. The majority of ETH is metabolized and rapidly excreted in the urine.[35] A substantial amount is converted to its sulfoxide VI which has now been shown to be equally active and interconvertible with ETH in man and other species.[36,37,38] Extensive metabolism to inactive compounds also occurs by dethiation (directly or, more probably, through the sulfoxide) to the carboxamide, deamidation leading to 2-ethyl isonicotinic acid, and by 1-methylation to give VII, which is partly converted to VIII. Dethiated VII and VIII and the sulfoxide of VIII were also observed.[38]

Continued work on sulfanilamides includes studies on tests to guide their use, new uses, new compounds, metabolism, protein binding, and a toxicity warning. An improved method for determining bacterial sensitivity to sulfa drugs permits reliably taking advantage of their activity against 80-90% of E. coli and Proteus mirabilis urinary infections in general practice, in bacteriuria of pregnancy and in hospital patients.[39] Synergism between the ineffective polymyxins and the bacteriostatic sulfa drugs in vitro, using a number of strains of Proteus mirabilis and vulgaris, led to an 8- to 64-fold decrease in active concentration and to bactericidal action[40,41] (cf. in vivo work in 1963). No such interaction occurred with either of these agents when combined with five antibiotics.[40] Plasmodium falciparum strains resistant to chloroquine and quinine are clinically susceptible to sulfanilamides and diaminodiphenylsulfone.[42,43] Sulfasymazine "appears to be a useful agent in the treatment of bacteriuria during pregnancy."[44] Using the computer method recently reported, the

dosing interval and the maintenance dose for sulfasymazine has been calculated.[45] As in other sulfanilamido heterocycles, introduction of bromine or chlorine into the heterocycle increased the persistence[46] of 4-sulfa-2,6-dimethoxypyrimidine; acute toxicity and protein binding were also increased. The isomeric 5-sulfa- 3-ethyl-1,2,4-thiadiazole and 2-ethyl-1,3,4-thiadiazole were compared[47] as to detailed differences in renal elimination characteristics independent of protein binding. Clinical and pharmacological studies were done on extremely long-acting 4-sulfa-5,6-dimethoxypyrimidine,[48] and on this and four other long-acting sulfa drugs (3-sulfa-6-methoxypyridazine, 4-sulfa-2,6-dimethoxypyrimidine, 2-sulfa-5-methoxypyrimidine and 2-sulfa-3-methoxypyrazine).[49]

A study[50] of 2-sulfanilamidopyrimidine itself rounds out the picture of the metabolism of sulfapyrimidines:[51] large variations in $N^4$-acetylation, in $N^4$-sulfonate, $N^4$-glucuronide and $N^1$-glucuronide formations occur in closely related compounds. The 5,6-dimethoxy- and 2,6-dimethoxy- 4-sulfapyrimidines are 2% and 80% converted to glucuronides. In contrast to these and other sulfapyrimidines, 2-sulfa-4,6-dimethoxypyrimidine is not $N^4$-acetylated in vivo; its $N^4$-acetyl derivative is, in fact, rapidly deacetylated.

Further study of sulfa drug protein binding has indicated that there is more to its effect on antibacterial activity than just the percent bound at equilibrium under special conditions. Human serum was found to increase the minimal inhibitory concentration for both sulfadiazine and sulfadimethoxine but the increase for each was eight times as great for Shig. dysenteriae as for E. coli.[52] In addition, the ratio of the increases in m.i.c.'s of the two sulfas in the presence of 20-50% human serum was too small (2) to correspond to the ratio (42) of percentage of unbound sulfa. The binding capacity of human serum protein determined by equilibrium dialysis[53] was nearly the same (ca. 2 moles/mole protein) for 8 sulfanilamides ($pK_a$'s 5.7-7.2), this small capacity supporting chemical rather than absorptive binding; protein affinities varied a hundred-fold. A quite different number (70) of binding sites for one of these, sulfamethoxypyridazine, was calculated[54] from thermodynamic data based on equilibrated Sephadex columns; the energetics indicated binding by van der Waal's forces.

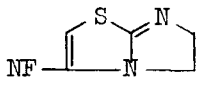
A warning has been issued to physicians that the long-acting sulfanilamides, sulfamethoxypyridazine and sulfadimethoxine, have been implicated in the development of Stevens-Johnson syndrome.[55] Over the years, many other drugs and certain conditions (vaccination, foods, infections themselves) have also been implicated,[55,56] and this very low incidence syndrome may be an allergic reaction with multiple etiology or may have a single, still undetermined cause.[56]

Quinoxaline di-N-oxides, which were extensively investigated 15-20 years ago as antiviral, amebicidal and antibacterial agents, have received some further attention. Compounds such as 2,3-dimethylquinoxaline-1,4-dioxide were shown[57] earlier to be active as the result of rapid metabolism to the hydroxymethyl analog. Recent work[58] has disclosed also the bis(hydroxymethyl) metabolite, which was still more active. However, the toxicity increased as did the activity and precluded chemotherapeutic use.[58] The mono and bis acetoxymethyl analogs were found[59] to have reasonable Gram-negative activity (50% survival at 20-120mg/kg intraperitoneally) in mice but near toxic levels. A quinoxaline-1,4-dioxide-2-aldehyde hydrazone (R—CH=N-oxaz-

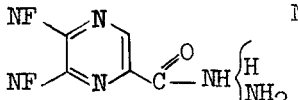
olidin-2-on-3-yl), orally active against Pasteurella in mice ($ED_{50}$ 13mg/kg) and in a Proteus urinary infection model in rats (min. effective dose 3mg/kg),[60] is possibly related more to its nitrofurfurylidene analog, furazolidone, than to the dioxides above, since the active series here comprises only hydrazones. No toxicity relationship to either was indicated.

Three recent papers[61,62,63] reported the activity of three ammonium bis-quaternaries, $ZCH_2(Me_2)\overset{+}{N}C_6H_{12}\overset{+}{N}(Me_2)CH_2Z$ where Z is $C_{11}H_{23}$, $COOC_{12}H_{25}$ and $COOC_{10}H_{21}$, against a lethal Klebsiella rhinoscleromatis infection in mice, intraperitoneal dosage of 2-5mg/kg twice daily for 12 days giving 100% survival. The $LD_{50}$ single dose values given were 70-100mg/kg and no data or comment on activity against other organisms was given.[61]

Antiseptics (In Vitro Actives).—Compounds are included here if reasonably complete experimental in vitro data have been reported—inactivity in vivo is assumed unless the article states otherwise. For assessing practical applicability, the effect of protein and lipid on in vitro activity and toxicity data are necessary but are frequently lacking. In vitro structure-activity studies on in vivo actives are covered in that section. No in vitro antituberculous reports have been included.



IX



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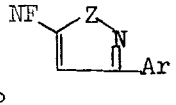

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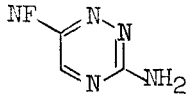
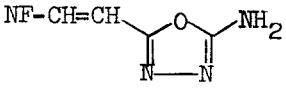


XII

The bicyclic IX (furazolium chloride as its salt) had broad-spectrum activity[64] (0.4-12mcg/ml) substantially superior to that of nitrofurazone and comparable to that of nitrofurylacrylamide. C-Methyl substitution or ring expansion decreased activity while insertion of a vinyl group increased activity against Staph., Strep. and Aerobacter. The bis(nitrofuryl) X had Coli-Staph. activity comparable to IX but a narrower spectrum.[65] Some aryl and heteroaryl pyrazoles and isoxazoles XI were about as active[66] (0.2-10mcg/ml) as those reported earlier. The as-triazine XII had broad activity[67] (0.2-1mcg/ml) but apparently not as broad as the vinyl analog,[68,69] or the 3-$N(CH_2OAc)_2$ derivative of the vinyl analog.[70]



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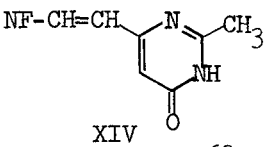
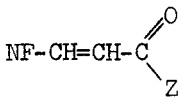
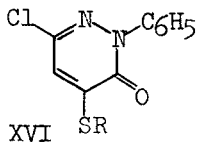


XIV



XV



XVI

Further work on nitrofuryl vinyl[68,70] and butadienyl compounds has been reported. 3-Amino-6-[3'-methyl-4'-(5-nitro-2-furyl)-butadienyl]-as-triazine, the butadienyl analog of XII, was very active in vitro (Coli-Staph. at 0.03-0.06mcg/ml).[71] In 2-disubstituted amino-1,3,4-oxadiazoles, the nitrofuryl-3'-methylbutadienyl compound was less active than the vinyl analog, and the thiadiazole analog of the best (0.8-2mcg/ml) compound was less active.[72] Branching of the butadiene, especially with alkyls larger than methyl, decreased activity[73] of the 2-$R_2N$-oxadiazoles, as did a 2-alkyl or phenyl group on the oxadiazole.[74] 4-Methyl-3-methylthio-5-(5'-nitro-2'-furylvinyl)-1,2,4-triazole[75] had Coli-Staph. activity at 1-12mcg/ml.

A ten-fold increase in Coli-Staph. activity (to 1-2mcg/ml)[76] resulted on cyclization of the acyl semicarbazide to the 2-amino-oxadiazole XIII. The 2-oxo analog had Coli-Staph. activity at 1mcg/ml while its 2-ethoxy and

2-chloro analogs were much less active. Various 3-acyl-2-oxo analogs of XIII were equally active but less toxic;[77] the 3-$CH_3$-2-oxo analog was more active (Coli-Staph. at 0.1mcg/ml).[78] The 3'-methylbutadienyl 2-oxo analog[79] shifted in antibacterial spectrum (Staph. 0.4 and Coli 50mcg/ml). The pyrimidinone XIV was broadly active but only at 10-30mcg/ml except for Shigella (0.5mcg/ml); cross-resistance with furazolidone was not observed.[80]

In the 3-(5'-nitro-2'-furyl)acryloyl group XV, activity was poor when Z was pyrazoline, alkyl or carbethoxyalkyl.[81,82] When Z was 2-imidazoline, Staph. activity was lost without change in Coli activity relative to the simple amide (Z = $NH_2$).[83] When bromine is present (Z = $CH_2Br$ or NF—CH=C(Br)—CHO),[84,85] the compounds act by a different mechanism and are unstable in vivo to SH groups. When Z was NHNH-disubstituted pyrimidinyl,[76,86] Coli-Staph. activity was good (1mcg/ml) but not when Z was NH-5-$CH_2$pyrimidinyl-2,4-$R_2$ (10mcg/ml)[86] or NHNH-certain acyls.[87] The spectrum of 60 nitrofurans was studied:[88] classes of compound in decreasing order of activity are NF—CH=CH—Z, NF—CH=N—NH—hetero, NF—CH=N-hetero, NF—hetero, NF—C(=NH)—NHNHR.

In the aldoxime, the aldehyde guanylhydrazone and vinyl-quinoline comparisons[89] of Coli-Staph. activity in vitro, changing 5-nitro- to 5-methylsulfonyl- furyl produced 4- to 100-fold decreases in activity, in contrast to the same variation on chloramphenicol.

The susceptibility of almost all strains of various species of Neisseria (gonorrhoeae, meningitidis, flava) to in vitro inhibition by acetazolamide (2-acetamino-1,3,4-thiadiazole-5-sulfonamide) is unique relative to other organisms,[90,91] but apparently does not carry over to in vivo infections. Inhibition of only N. gon. occurred at high $CO_2$ concentrations; the other N. and resistant bacteria appear to have low concentrations of carbonic anhydrase.[90]

A miscellaneous group of antiseptics reported is mostly reminiscent of known antibacterial types: 2-guanidino-6-substituted quinazolines[92] vs. Staph. and Salmonella at 10-100mcg/ml; N,N'-di(cyclohexylpropyl)-1,4-bis (aminomethyl)cyclohexane[93] vs. Staph., Strep., Salm. and Coli at 3-10mcg/ml; 3-(p-bromophenyl)-6-bromo-1,3-benzoxazine[94] vs. Salm. and Klebsiella at 20-40mcg/ml; and sodium dodecyldi-(and mono)chlorodiphenyloxidesulfonate[95] vs. Staph. at 5mcg/ml. The activity of the pyridazinones[96] XVI (R = H and $CH_3$) is curiously specific: 0.5-3mcg/ml against Shigella species but much less effective against Coli and Salmonella.

Mechanism of Action.—Nalidixic acid I selectively inhibits DNA synthesis in growing E. coli, acting directly against its replication.[97] The bactericidal phase of its action can be prevented by inhibition of the synthesis of protein (e.g. by chloramphenicol) or of RNA. Similarly, the addition of several, but not all, nitrofurans antagonized its in vitro antibacterial activity against various Proteus-Providence species,[98] but nalidixic acid did not oppose the action of the nitrofurans. The latter also antagonized its activity against Coli and Salmonella strains,[99] as did tetracycline and chloramphenicol against a number of coliform organisms.[100] Against B. subtilis, one of the few Gram-positive bacteria susceptible to it, the mode of action is also selective inhibition of DNA synthesis.[101] The various hypotheses on the mechanism of action of isoniazid have been reviewed.[102]

Research Techniques And Screening Methods.—A variety of chromatographic techniques continue to be applied to separation and identification of syn-

thetic antibacterial agents and their metabolites. Gas chromatography of 60 nitrofuryl and nitrofurylvinyl heterocycles has been accomplished[103] on a silicone column generally without decomposition. Nitrofurantoin, nitrofurazone, and furazolidone as well as a large group of other nitrofurans were studied in a variety of solvent systems by thin-layer[104] and by paper[105] chromatography.

About 20 sulfanilamide derivatives have been characterized by their thin-layer chromatographic behavior in a dozen solvent systems($CHCl_3$-ROH mixtures with or without a third solvent) using detection with iodine,[106] p-dimethylaminobenzaldehyde,[107] or $CuSO_4$-$NH_4OH$[108] and quantitative ultraviolet spectrophotometry.[106,109] A two-dimensional thin-layer qualitative method using a basic and an acidic system was also reported.[110] Work continues[111] on automated analysis of sulfa drug samples (20 per hour) based on the usual diazotization and coupling method; reproducibility for therapeutic concentrations was satisfactory (1-2%). Paper chromatography of tuberculostatic agents containing a C=S moiety employed rapid development with methanol-water and detection by means of iodine-azide or mercury-fluorescein reagents.[112] Differential thermal analysis has been applied to the examination of the identity and purity of ethambutol, sulfa drugs and other synthetic pharmaceuticals.[113]

Analog computers were used for model calculations on the influence of protein binding on the clearance of a long-acting 4-sulfa-5,6-dimethoxypyrimidine[114] and for devising optimal therapeutic regimens for sulfasymazine.[45]

No new antibacterial screening methods were reported. Recent success in experimental animals and in vitro with growing the leprosy bacillus has led to significant and promising additions to our knowledge.[115] Over 100 Myco. leprae strains from all over the world and from the several clinical types of leprosy have now produced mouse foot-pad infections. The relationship of bacterial morphology, administration of antileprotic agents, BCG vaccination, and immune-response suppression to production of increased infectivity in mice has been studied. The neural involvement produced by this organism in the mouse and hamster infections is unique among the mycobacteria and is characteristic of the clinical disease.

Reviews.—The following have been reviewed: species differences among dihydrofolate reductases,[116] ethambutol laboratory and clinical studies,[29,117] structure-activity relationships in ethambutol analogs,[32] mode of action of isoniazid,[102] progress in leprosy research,[115] long-acting sulfanilamides in urinary tract infections,[49] and infectious drug resistance.[1,2,3,4]

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# Chapter 13. Antiviral Agents

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Because it is brief, this review regrettably must exclude certain significant aspects of antiviral research. No attempt will be made to include the substantial basic research on viral inhibition. Perhaps even more serious is the exclusion of papers on interferon and agents that induce interferon (statalon, helenine). It is possible that interferon is the key to a comprehensive approach to the problem of human viral disease but more work must be done to establish that interferon has a role in recovery from such disease in humans.

At least one review appeared in 1966.[1] It is rather unfortunate that the authors' introductory remarks indicate that basic research has now established the feasibility of specific viral inhibitors. Basic research has only confirmed what has been known for well over a decade: that viruses can be inhibited without harm to the host. Perhaps the basic researcher has been slow in capitalizing on early antiviral studies. It is worth noting that virtually all agents presently used in fundamental antiviral studies were discovered in less than a logical manner--in fact, most were products of screening programs. The authors imply that there was logic to the discovery of hydroxybenzylbenzimidazole; actually, this was first observed to be an inhibitor of poliovirus in 1955 in the course of a screening program.[2] The authors may well have later rediscovered this compound but apparently only after screening a "large number" of benzimidazole derivatives. Such historical reflection seems necessary at times to establish how antiviral agents have been discovered.

For the present, perhaps the term "chemotherapy" should be replaced by "antiviral." Few seem clear in their use of the term "chemotherapy" in viral disease. Indeed, in that some of the most interesting antiviral drugs are protective only, the term "chemotherapy" is completely inaccurate.

Adamantanes. Amantadine hydrochloride (Symmetrel, EXP 105), now released by the Food and Drug Administration, has been further examined in double-blind studies in volunteers infected with influenza $A_2$ virus[3] and in epidemics caused by the same virus in adults[4] and in children.[5] These studies noted no significant toxic reactions despite prolonged dosage schedules. All studies indicate some suppression of antibody response to the infecting virus and a substantial decrease in the number and severity of influenza cases. The drug did not completely suppress the antibody response or the disease. Apparently only one trial

included attempts to isolate the virus.[5] Although there has not been a high degree of success in using virus shedding as a parameter in this type of clinical trial, there was considerable success with this technique in an experiment with horses infected with influenza A/EQUINE 2 virus.[6] Only three of seven horses treated with amantadine briefly shed virus while all seven untreated animals shed virus for 8 days. Drug-treated horses had a decreased immune response but it was thought that they were immune to rechallenge with the same virus. There has been some concern that a highly active prophylactic agent might prevent development of natural immunity. However, even if this is a genuine problem, amantadine is clearly not sufficiently potent. In mice infected with an adequate dose of virus, amantadine suppressed but did not eliminate the specific antibody response.[7] Amantadine apparently does not have an effect on antibody response in general.

One report[8] indicates that amantadine, ammonium ion, and caprochlorone may have the same mode of action: they inhibit penetration of influenza virus into the cell. This further emphasizes the usefulness of drug research in exposing a previously little known aspect of cell-virus interaction. There remains, however, a continuing problem of quantitation in comparative studies of antiviral agents. There is no general agreement on which tests are valid in demonstrating an antiviral effect and which tests produce reliable quantitative data. Some effort is being made to study the proper experimental design in tests with mouse influenza and amantadine.[9] Still another in-vitro antiviral test, a copy of the gradient-plate method used in antibacterial studies, has been described.[10]

Both amantadine and rimantadine hydrochloride (EXP 126, α-methyl-1-adamantanemethylamine HCl) were given to children during an epidemic caused by measles and influenza B viruses.[11] No beneficial results were observed, which is not surprising because these two viruses are not very sensitive to these drugs but it is of interest that rimantadine is being tested clinically. This compound is about eight-fold less toxic in embryonated chicken eggs than is amantadine, but it may be slightly more toxic in mice.[12] In mouse experiments rimantadine is quantitatively superior to amantadine against influenza A virus infections, and it showed stronger therapeutic effect. More than 1,200 humans have received the drug and it appears to be better tolerated than amantadine.[13] Preliminary clinical trials indicate that rimantidine is active in protecting against influenza $A_2$ virus disease.

N-Methyl-1-adamantanecarboxamide octochloro chlorination product was reported[14] to have a remarkable curative effect in mice infected intracerebrally with lethal doses of neurotropic influenza A/WSN virus. Even if treatment is withheld until the mice show tremors and paralysis, they can still be saved; however, this drug has no effect on pulmonary influenza virus infections in mice. Whatever its use, this drug certainly manifests a true chemotherapeutic action.

It is only fair to point out that, with protective drugs active against only certain strains of influenza virus, there are substantial logistic problems. Before amantadine can be used, there must be timely laboratory diagnosis indicating that a community is under attack by a virus which is sensitive to the drug. Few physicians are in a position to obtain such timely information. Suggestions have been made on how the entire problem of more rapid laboratory diagnosis might be approached.[15]

Thiosemicarbazones. A number of reports on the use of methisazone (N-methylisatin-β-thiosemicarbazone, marboran, 33T57) appeared in 1966. This drug was found to be beneficial in vaccinia gangrenosa,[16] ineffective in chickenpox[17] [not surprising], rather effective in protecting humans against variola minor (alastrim),[18] beneficial in malignant lymphoma in man[19] [which, if confirmed, would be remarkable], perhaps useful in children given smallpox vaccine when it is contraindicated,[20] beneficial in a serious case of eczema vaccinatum,[21] and inadequately tested in a unique case of cowpox.[22] In virtually all these clinical trials there was a substantial amount of nausea and vomiting. Even with this troublesome reaction it would seem wise to have the drug available in the United States for use in the occasional smallpox vaccine reactions, which are often fatal, and the rare smallpox scares.

On the basis of development of resistance to methisazone by rabbitpox virus in tissue culture and in rabbits, the possibility of this problem also occurring in the use of this drug in smallpox was suggested.[23] As shown in many antibacterial studies, in-vitro induction of resistance does not necessarily have clinical implications. There is some question whether use of this drug will ever become so extensive that drug resistance could occur. During 1966, two reports appeared on test methods for thiosemicarbazones, one involving vaccinal tail lesions in the mouse[24] and the other a combined use of anticonvulsants with antiviral compounds to control vaccinia virus infections in mouse brain.[25]

Inhibition of adenovirus 11 by methisazone[26] came as a surprise; however, the virus dose used was extremely small and it was found, by other test methods,[10,27] that methisazone did not affect plaque formation of adenoviruses 1, 2, and 5.[28] These same methods did reveal that methisazone produced considerable toxicity in HeLa cells, the type of cell used in the original study. It is possible that the antiviral action observed was due to a toxic reaction not readily observed in the test system used.

Extensive clinical trials were made with isothiazole thiosemicarbazone (4-bromo-3-methyl-isothiazole-5-carboxaldehyde thiosemicarbazone, M & B 7714) which, although very active in animal tests, has virtually no activity against smallpox either therapeutically or prophylactically.[29,30] Again vomiting was a common reaction, and this drug also produces an unconjugated hyperbilirubinemia in humans, which has now been studied in rabbits.[31]

Nucleosides. Apparently 5-iodo-2'-deoxyuridine (IUdR, idoxuridine) has become a standard drug for treatment of herpetic eye infections. The most recent report of its effectiveness in this disease involved 1,202 human cases.[32] There has been some concern about IUdR inhibiting corneal healing; it now appears that, in herpes simplex infected eyes, corneal healing is not reduced.[33,34] Further studies in hamsters have also reconfirmed the therapeutic effectiveness of this drug.[35]

Some very encouraging results have been obtained in the treatment of dermal herpetic lesions in man with IUdR.[36,37] If the drug was dissolved in dimethylsulfoxide (DMSO) it was especially active; indeed, DMSO alone seemed to be beneficial. Both of these studies were well controlled, and in one of them both virus isolation and serologic tests were used to confirm the diagnosis. Early use of the drug on the involved tissue is essential. Because herpes simplex virus is sensitive to many compounds, the treatment of dermal herpes seems long overdue. The Upjohn Company has been pursuing such a clinical trial using the antiherpetic compound cytosine arabinoside (cytarabine, CA).[38]

It has been claimed that cytarabine has some toxicity in the cornea when used in herpes keratitis; however, a recent report indicates that this so-called toxicity is essential to the antiviral effect of the drug.[39] There is also a report of successful treatment of a single case of vaccinial blepharokeratitis[40] with cytarabine. There have been a number of reports on the inhibition in tissue culture of DNA-containing adenoviruses by cytarabine.[41] With other test methods[10,27] it was found that DNA inhibitors, such as IUdR, BUdR, and CUdR, were not highly active against adenoviruses 1, 2, and 5, and this is true also of cytarabine which was much more toxic than the rest.[28] Some antiviral activity could be demonstrated, especially with CUdR, but it was not remotely comparable to that seen with herpes simplex and vaccinia viruses. It has been surprising that DNA-containing adenoviruses seem to be resistant to such inhibitors.

IUdR was found to be active against infectious bovine rhinotracheitis virus in cell culture but there was some doubt about activity in rabbits.[42] Trifluorothymidine seemed to be more effective than IUdR in herpetic corneal lesions in rabbit eyes,[43] while the antiherpetic compound methylaminodeoxyuridine was less effective than IUdR in the same study.[43]

In cell culture studies, 6-azauridine was much less active than IUdR against vaccinia virus,[45] and azathymidine and uracil methyl sulphone were less active against herpes simplex virus.[46] 9-Cyclopentyl-6-mercaptopurine was claimed to be active against Friend leukemia virus in vivo.[47] Of interest is the antiviral effect found for $N^6$ (2-hydroxyethyl)-adenine in mice infected with Coe virus (coxsackie A-21).[48] Although this is not a typical common cold virus, it does produce colds in human volunteers (there has been a study of the effect of this drug in such volunteers but

the details have not been published). In mouse studies, antiviral effects were observed with one tenth the lethal dose of the drug. Drug toxicity could be suppressed by xanthine oxidase inhibitors which did not influence the antiviral action. It is significant that both animal and human trials can be used to study inhibition of this virus.

Other antiviral agents of interest. There have been reports of antiviral agents active in laboratory tests and it is not easy to determine which of these, if any, might lead to a useful drug. Some investigators may still be confusing toxic effects on the host with true antiviral action. Despite this, antiviral studies have made commendable progress from the original concept that specific viral inhibition was not possible.

A further report has been made on phagicin, a protein material from phage-infected Escherichia coli that has shown antiviral effects against vaccinia and herpes simplex infections of the eye.[49] Apparently this protein is a poor antigen. Hydroxyurea is an inhibitor of DNA synthesis in a number of biologic systems, including those of vaccinia and herpes simplex viruses.[50-52] Because viral protein synthesis appears to be unaffected, the authors suggest this might make a useful drug that would not influence specific immune response. This seems a little premature in the absence of substantial proof of activity in vivo.

In further studies of plant antiviral substances it was found that a number of extracts had activity against influenza virus in cell cultures and in mice.[53] Influenza A virus appeared to be inhibited in mice treated with 1-(4-fluorophenyl)-1-phenyl-2-propynl-N-cyclohexyl-carbamate (FPC)[54] and 2-diethylaminoethyl-4-methylpiperazine-1-carboxylate.[55] The latter compound, when studied in cell culture, appeared to have a mode of action much like that of amantadine.[56] Compounds that stimulate mucus production (cholinergic compounds) made influenza A virus infection in mice more severe while anticholinergic drugs made the disease less severe.[57] The concept of using pharmacologically active drugs rather than specific antiviral agents has some merit and certainly requires further exploration, especially for the problem of the common cold.

Viractin, a distillate from Streptomyces griseus, has been claimed to protect humans from respiratory virus disease. It was found, however, that the material had no in-vitro or in-vivo activity in tests against a variety of respiratory viruses.[58] A note appended to this report is highly critical because apparently no claim was made for activity except in humans. Products from E. coli,[59] propionibacteria,[60] and Salmonella typhosa[61] have produced interesting antiviral effects. It must be said, however, that the search for antiviral antibiotics is still in its infancy.

Among a host of other papers on antiviral effects is one reporting that huge doses of the plasma expander, polyvinylpyrrolidone, inhibited vaccinia and herpes simplex viruses in cell cultures.[62] Substituted

morpholonium quaternary compounds have been shown to be active in mice infected with mouse hepatitis and herpes simplex viruses.[63] In cell culture, $N^1$-furfurylbiquanide was active against a variety of RNA viruses.[64] A lengthy report on the effect of salicylates on EMC virus in cell culture is not completely convincing.[65] Certain derivatives of 4-oxo-5-thiazolidine are claimed to have a wide antiviral spectrum but only data for poliovirus inhibition are presented.[66]

Bauer[67] has made an interesting suggestion in attempting to relate virus size to the effectiveness of antiviral agents. It is perhaps somewhat early in the history of the search for antiviral drugs to draw too many conclusions from the presently known antiviral substances. One wonders what the conclusions would have been if a few dozen other known antiviral agents had been incorporated in Bauer's study. Various pharmaceutical companies still have a number of agents not yet reported in the literature; for example, Smith Kline & French Laboratories has a compound or compounds highly active against a number of rhinoviruses in cell culture systems.[68] Without information on a number of such compounds, analysis at this time seems premature.

An interesting and comprehensive study of compounds that release cyanate in vivo, resulting in antiviral effects only against pseudorabies virus,[69,70] illustrates the fundamental discoveries resulting from studies of agents uncovered in a screening program. A close look at the products of screening programs could convince one that, although these programs have been the sole source of antiviral drugs so far, they in fact also may have been even more productive in basic research.

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# Chapter 14a. Human Antiparasitic Agents

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INTRODUCTION — Better drugs are still needed for the treatment of many chronic and debilitating human parasitic diseases, including schistosomiasis, filariasis, Chagas' disease, leishmaniasis, trichuriasis, and strongyloidiasis. Moreover, a reappraisal of the status of malaria chemotherapy has indicated: (1) the urgent need for developing new fast-acting suppressive agents effective against drug-resistant plasmodia; (2) the potential usefulness of long-acting drugs, particularly in malaria eradication programs; and (3) the importance of developing safe and effective anti-relapse drugs capable of effecting a radical cure in a single dose or, at most, in a three-day regimen. During the past year significant progress was made in man's continuing struggle against parasitic diseases. In the discussion that follows, frequent reference is made to recent reviews[1-5] that provide background information on broad aspects of parasite chemotherapy.

MALARIA — Several reviews have recently been published.[1,3,5-8]

(a) Experimental Models for Studying Drug-Resistance — The problems posed by the existence of drug-resistant strains of falciparum malaria have spurred efforts to develop better experimental models for studying this phenomenon. As reviewed elsewhere,[5,9] strains of avian, murine, and/or simian parasites have been induced to acquire resistance to representatives of all the major classes of antimalarial drugs, and the resistant lines have been employed in cross-resistance studies to determine the interrelationship among the various chemical types.[3,5,10-15] Results from these experiments provide a better rationale for selecting alternative drugs for use against drug-resistant parasites, and afford a basis for recognizing compounds with a novel mode of action.

(b) Mode of Action of Antimalarial Drugs — It is generally assumed that antimicrobial agents exert their effect by inhibiting essential enzymatic reactions in the affected microorganism itself. Among intracellular organisms such as malarial parasites, however, the possibility exists that the agent inhibits an enzymatic reaction of the host cell that produces a product essential to the parasite. The latter mechanism is apparently involved in the action of anti-pantothenates against Plasmodium lophurae. This parasite, which requires an external source of coenzyme A (CoA), is inhibited by anti-pantothenate (SN-14,622) when it is developing intracellularly, but not when it is cultured extracellularly in vitro.[16] The anti-pantothenate presumably interferes with a red-cell system synthesizing CoA, thereby depriving the parasite of an adequate source of CoA.

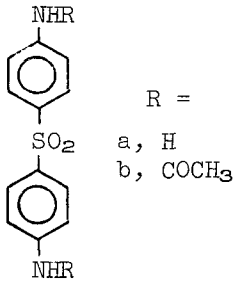
A recent comparison of the action of chloroquine, quinacrine, pyrimethamine, and sulfadiazine on P. lophurae developing intracellularly or extracellularly in vitro showed that each drug had the expected inhibitory effect on

parasites developing within their host erythrocytes, but that none inhibited the development of extracellular parasites at the same concentrations.[17] Assuming that parasite integrity is maintained, one explanation would assume that certain drugs are concentrated in the red cells so that parasites within the cells are actually exposed to much higher concentrations than were used in the medium. This explanation is plausible with regard to chloroquine; for recent studies[18] suggest that chloroquine is selectively toxic because it attains higher concentrations in parasitized red cells than in normal erythrocytes, and that resistance is due to an impairment of the mechanism by which such drug levels are accumulated. A second explanation is based by analogy on the proposed mechanism of action of the anti-pantothenates, and would assume interference by the drug with a host cell-enzyme system that supplies a product required by the parasite.[17]

A number of considerations place P. berghei in the class of microorganisms that are dependent on endogenous synthesis for folate-containing cofactors.[19,20] Recent experiments show that P. berghei can convert dihydrofolate ($FH_2$), but not folate, to materials capable of satisfying the growth requirements of Pediococcus cerevisiae, and therefore presumably cofactor forms of tetrahydrofolate ($FH_4$).[20] This shows that cells of P. berghei are capable of folate cofactor biosynthesis and do not have to depend on the host erythrocytes for this function. The utilization of $FH_2$ required NADPH and was inhibited by pyrimethamine,[19] which suggests the presence of $FH_2$ reductase in these parasites.[20]

(c) Suppressive Antimalarial Drugs

(1) Sulfones and Sulfonamides, either singly or in combination with pyrimethamine, are of considerable interest as possible alternative drugs for oral suppressive use against drug-resistant P. falciparum. Among them, diaphenylsulfone (DDS)(Ia), conventional sulfonamides such as sulfadiazine, and the long-acting congeners sulforthomidine (IIa), sulfamethoxypyridazine (IIb), sulfapyrazinemethoxine (IIc), and sulfadimethoxine (IId) have been evaluated.[3,5,21-27] In man, response to these drugs is characterized by

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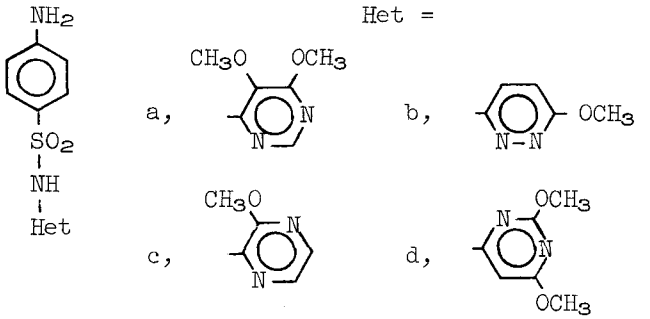


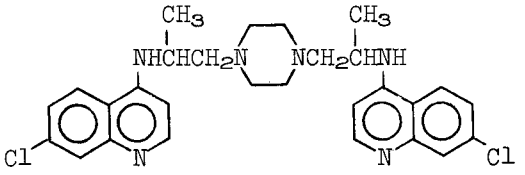

II

relatively slow antimalarial action,[5,21-24] greater efficacy against P. falciparum[21-25] than P. vivax,[26] suppressive action against multi-resistant

P. falciparum,[21,23,27] and good tolerance.[21-27] The daily administration of 25 mg of DDS concurrently with 300 mg of chloroquine base and 45 mg of primaquine weekly proved highly effective in preventing patency following challenges with two strains of chloroquine-resistant P. falciparum.[27]

The sulfone-sulfonamide group acts at a different site than cycloguanil (IX) or pyrimethamine, presumably by preventing the incorporation of p-aminobenzoic acid into folic acid. Utilization of the two types in combination enhances antimalarial activity in experimental infections[3,5,15] and in man,[3,22,26] affords broad action against resistant organisms,[3,5,15] and decreases the likelihood of the emergence of resistance.[3,5] There is undoubtedly a need for further assessment of the antimalarial value of sulfones and sulfonamides, both alone and in combination with other agents. Because of known deficiencies, however, they are not likely to provide the final solution to the problem of treating drug-resistant malarias.

(2) 4-Aminoquinolines – Earlier reports[3,28] that III was active against chloroquine-resistant P. berghei were surprising, since the chloroquine-resistant strains studied to date have proved uniformly cross-resistant to other 4-aminoquinolines and to 9-aminoacridines.[5,6] However, recent studies utilizing a different chloroquine-resistant strain of P. berghei showed a high degree of cross-resistance between III and chloroquine.[29] Similarly,



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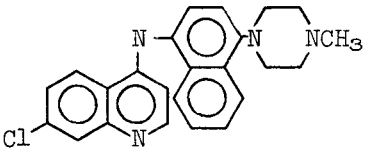
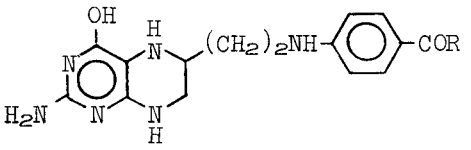


IV

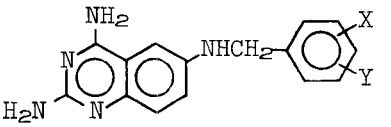
IV and relatives exhibited potent activity against normal P. berghei, but were ineffective against a chloroquine-resistant strain.[30]

(3) Potential Antimetabolites – Several new types are reported to be active against drug-resistant malarial parasites in experimental animals. Tetrahydrohomopteroic acid (Va) was effective against both normal and pyrimethamine-resistant P. cynomolgi in monkeys,[31] but tetrahydrohomofolic acid (Vb)



R = a, OH; b, $NHCH(CO_2H)(CH_2)_2CO_2H$

V



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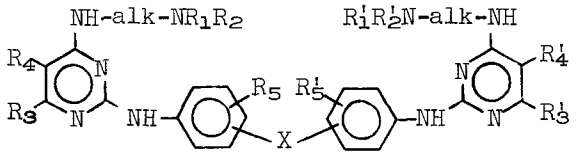
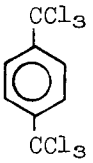


VII



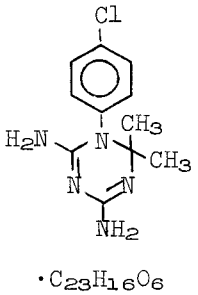
VIII Hetol®

was inactive.[31] Activity was also reported among certain 2,4,7-triamino-6-phenylpteridines,[32] 2,4-diaminoquinazolines (VI),[33] and bis(2,4-diaminopyrimidines)(VII).[34]

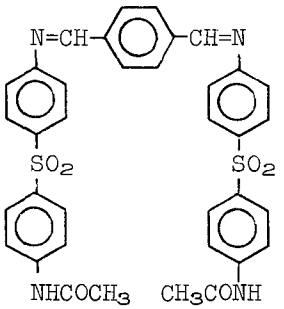
(4) Other Agents — Prodigiosin,[35] 1-aminocyclopentane carboxylic acid,[36] and Hetol® (VIII)[36] are effective against P. berghei in mice. Hetol® is also active against P. cynomolgi and P. knowlesi in the monkey.[36]

(d) Repository Antimalarial Drugs

(1) Dihydrotriazine and Pyrimethamine Salts — A single 5 mg/kg I.M. dose of cycloguanil pamoate (Camolar®)(IX) protects man for many months against challenges with susceptible strains of P. falciparum or P. vivax.[3,37,38] Urinary excretion studies in human volunteers indicate that protection is maintained until the daily cycloguanil excretion rate decreases to 0.25 mg or less, usually for 8-9 months.[38] In accord with theoretical expectations, the drug was ineffective against parasites known to be highly resistant to chlorguanide, pyrimethamine, or both.[3] Cycloguanil pamoate is useful, however, against partially resistant strains, although the protection period is shortened.[39,40] Further, coadministration of amodiaquine with cycloguanil pamoate prolonged the period of protection in areas where parasites have some degree of resistance to chlorguanide or pyrimethamine but are sensitive to the 4-aminoquinolines.[41] Cycloguanil pamoate was well tolerated with respect to systemic toxicity and provoked only mild to moderate local reactions at the injection site when injected properly.[3,37-41]



IX Cycloguanil



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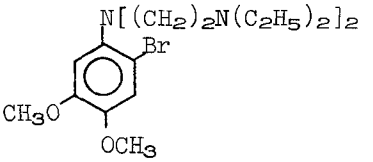


XI RC-12

(2) Sulfones — Potential long-acting sulfone and sulfonamide derivatives have been synthesized[3,42,43] to provide substances that (1) in combination

with cycloguanil pamoate or related compounds[3,44] might enable a sequential block in the metabolic pathway and afford broader repository action against drug-resistant lines than either drug alone, and (2) may be particularly convenient in the treatment and prophylaxis of leprosy.[42,43,45] Among them, DADDS (Ib) has been studied most extensively. A single parenteral dose of DADDS protected mice for many weeks against challenges with P. berghei and monkeys for many months against challenges with P. cynomolgi trophozoites or sporozoites.[3,42,46] CI-564, a mixture of cycloguanil pamoate and DADDS, showed better activity than either component alone against drug-resistant plasmodia in animals[46] and in man.[40]

The duration of action of PAM-1503 (CI-608)(X)[43] and six other repository sulfones against P. berghei in mice was intermediate between the short-acting DDS and the very long-acting DADDS.[47] Generally, the pattern of urinary excretion in rats also was intermediate between that of DDS and DADDS.[47] While additional work may be needed to assess the best form and amount of repository sulfone for use with cycloguanil pamoate, the potential antimalarial value of such a combination has been demonstrated.[3,40,42,46] Further, each of these compounds, together with DADDS, completely suppressed Mycobacterium leprae infections in mice when given in single parenteral doses at 2-month intervals.[45]

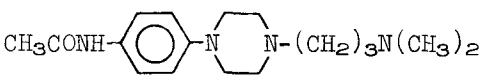
(e) Radical Curative Agents — RC-12 (XI) has substantial activity against the preerythrocytic forms of P. cynomolgi in the monkey but shows little promise as a schizonticidal or suppressive agent.[48] No reports have yet appeared concerning its efficacy and tolerance in man. Related compounds have recently been described.[49]

LEISHMANIASIS — Leishmania is responsible for millions of smoldering ulcers among inhabitants of endemic regions in Africa, the Americas, and the Near and Far East. Recent studies have confirmed earlier reports[3] that the repository antimalarial drug cycloguanil pamoate (IX)(supra) shows promise in the treatment of cutaneous leishmaniasis. Thus, 63 of 83 patients infected with Leishmania mexicanum were cured with a single dose of cycloguanil pamoate, including some subjects with a condition of more than 20 years' duration.[50] An effective repository drug would have important advantages over previous methods of treatment since dermal leishmaniasis occurs mainly among people in remote areas.

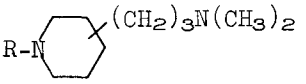
Several other compounds have recently been reported to be effective against leishmaniasis in experimental animals and in man, including monomycin,[51] paromomycin,[51] dehydroemetine,[52] and Berenil®.[53]

CHAGAS' DISEASE — At least 7 million persons in South and Central America are infected with Trypanosoma cruzi, the causative agent of Chagas' disease. In contrast to the successful development of effective drugs for the African trypanosomiases,[1] the treatment of cruzi infections has been generally unsatisfactory. There is evidence that nitrofurans may be effective against acute infections, but no adequate curative agent has been found.[3,5]

No new information is available concerning the usefulness of piperamide maleate (XII)[54] against T. cruzi infections. Certain piperidine relatives



XII Piperamide

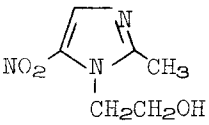


XIII

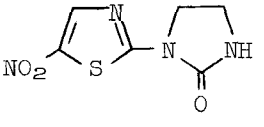
(XIII) are also effective.[55] Other compounds recently reported to show activity against T. cruzi in vivo include amphotericin B, emetine, isoquinoline-1-carboxaldehyde thiosemicarbazone, metronidazole (XIV), actinomycin D, and certain di- and triaminotriphenylmethane dyes;[56,57] 2,4-diaminoquinazolines (VI);[33] and quinazoline derivatives of aspartic and glutamic acids.[58] Actinomycin D, mitomycin C, and fluorouracil deoxyriboside inhibit the growth of T. cruzi in vitro by interfering with the synthesis of proteins and nucleic acids by the flagellate.[59] Furadantin sodium and NF-902 show early, marked action against intracellular forms of T. cruzi in chick embryo tissue culture.[60]

TRICHOMONIASIS — No parasitic infection is as common among women of the temperate zones as vaginal trichomoniasis.

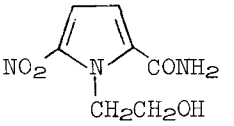
(a) Nitroimidazoles — Metronidazole (Flagyl®)(XIV) exhibits broad antiprotozoal activity and in particular has found widespread use in the oral treatment of trichomoniasis.[1,2] The metabolism of metronidazole in the dog and in man is similar.[61] In both species, the urine contained 61-69% of unchanged metronidazole, together with smaller amounts of the corresponding acid (26-28%) and a second metabolite which was tentatively identified as the ether glucuronide (5-11%). The authors found no evidence that the nitro group had been reduced. The synthesis of novel nitroimidazole compounds as potential antiprotozoal agents continues at a dizzying pace,[62-70] but none has yet been demonstrated to be superior to metronidazole against trichomoniasis in experimental animals or in man.



XIV Metronidazole



XV Niridazole



XVI 15,960 R.P.

(b) Other nitro heterocyclic compounds possess antitrichomonal activity, including niridazole (XV) and relatives,[71] nitrofurans,[72-74] nitropyrroles,[75,76] nitrothiophenes,[76,77] and nitropyrazoles.[78] Among them, 15,960 R.P. (XVI) is of particular interest. When given orally to mice, 15,960 R.P. was equi-active with metronidazole against Trichomonas vaginalis and nearly ten times as potent against Lamblia muris.[75] It also showed good oral activity against intestinal amebiasis in rats and hepatic amebiasis in hamsters due to Entamoeba histolytica. Urinary excretion studies in the

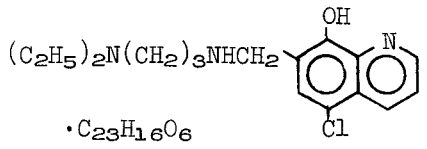
rat and dog were encouraging.[75] When 15,960 R.P. was given orally to rats in a single 250 mg/kg dose, 40% was recovered in the urine over a 48 hr period. Comparative recovery figures for metronidazole and aminitrozole[1,2] were 16% and 0.2%, respectively. In dogs, > 50% of the dose was eliminated in the urine within 24 hr, of which 70% was unchanged.[75] This suggests that 15,960 R.P. should reach the foci of infection in high concentration after oral administration.

AMEBIASIS – The ideal antiamebic agent must be capable of eradicating Entamoeba histolytica from both the bowel and from extra-intestinal sites and should be virtually non-toxic. Agents with sustained, prophylactic antiamebic properties are also needed. Although many good drugs are available, no single preparation is capable of exerting all the actions required.

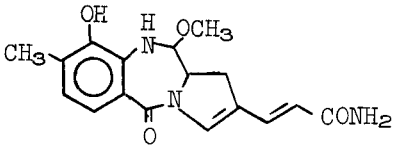
(a) 2-Amino-5-nitrothiazoles – Earlier studies[3,79] demonstrated that niridazole (Ambilhar®)(XV)[80,81] has strong therapeutic effects against amebiasis and schistosomiasis (infra) in experimental animals and in man. Results from recent human trials confirm that niridazole, administered in a dosage of 500 mg b.i.d. or t.i.d. for 10 days, is highly effective against both intestinal and hepatic amebiasis, but side-effects including frequent ECG changes and occasional neuropsychic episodes were encountered.[82-84] Although niridazole goes far toward meeting the therapeutic requirements of a single direct-acting amebicide, more extensive trials are needed to assess its safety in man relative to antiamebic agents in current use.[83-85] Many other 2-amino-5-nitrothiazole derivatives exhibit antiamebic activity.[71]

(b) Metronidazole (XIV) is ineffective against schistosome infections but is widely used in trichomoniasis (supra).[1,2] In a recent notable study, 800 mg of metronidazole given t.i.d. for 10 days cured 22 out of 25 patients with acute amebic dysentery, and each of 10 patients with confirmed amebic liver abscess.[86] In contrast to the niridazole studies, no significant ECG changes or neuropsychiatric disturbances were encountered in patients receiving metronidazole.[86] Further large-scale confirmatory trials are obviously necessary, but, on the basis of present results, the authors suggest that metronidazole may be unique as a single direct-acting amebicide, effective in the bowel and the liver, and without significant toxicity. Many other nitroimidazoles also exhibit antiamebic properties.[66,67,70]

(c) 8-Quinolinols – In recent clinical trials, clamoxyquin pamoate (Clamoxyl®)(XVII)[3] was compared with iodochlorhydroxyquin in the treatment of diarrheal disease.[87] Clamoxyquin pamoate was administered in a dose of 16 mg of clamoxyquin base per kg daily for 5 days. Iodochlorhydroxyquin was given in a regimen of 1500 mg daily for 10 days. Clamoxyquin pamoate was tolerated well and was equal to iodochlorhydroxyquin in reducing the daily number of stools, in improving stool consistency, and eliminating fetid odor. Both drugs were effective in eliminating blood and mucus when present in stools. Clamoxyquin pamoate was usually better than iodochlorhydroxyquin in ameliorating subjective gastrointestinal symptoms and was superior or equal to iodochlorhydroxyquin in eliminating E. histolytica, Giardia lamblia, and Shigella.[87]




XVII Clamoxyquin pamoate




XVIII Anthramycin methyl ether

(d) Anthramycin methyl ether (XVIII)[88] had a marked effect on intestinal amebiasis in the rat; single oral $CD_{50}$ values against the K9 and 200 strains of E. histolytica were 0.22 and 0.18 mg/kg, respectively.[89] The antibiotic also exerted a definite chemotherapeutic effect in experimental infections with Trichomonas vaginalis and Syphacia obvelata.[89]

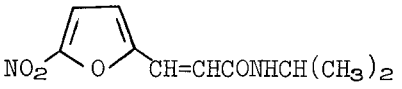
(e) Emetine inhibits protein synthesis in certain mammalian cells, species of Saccharomyces, and in Anemia phylitidis, a higher plant, but not in cell-free extracts prepared from E. coli.[90] Although different in chemical nature, (-)-emetine and the ipecac alkaloids show configurational and conformational similarities to (-)-cycloheximide and the glutarimide antibiotics and have similar effects on protein synthesis.[90] These results define a structural basis for the inhibition of protein synthesis, and suggest a novel synthetic approach to new compounds of potential interest.

SCHISTOSOMIASIS — Nearly 200 million people are infected with schistosomiasis, and on a world basis these infections are spreading. The degree of prevalence, the seriousness of the infection, and the paucity of adequate drugs make schistosomiasis one of the great challenges in parasite chemotherapy today.[2,3] Several reviews have recently appeared.[91,92]

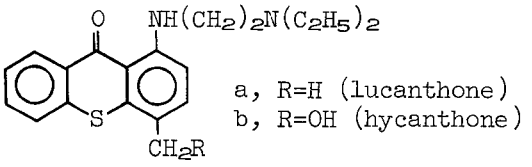
(a) 2-Amino-5-nitrothiazoles — Previous studies[3,79,84] indicated that niridazole (XV) was highly active against Schistosoma mansoni and S. japonicum in animals and showed promise against S. haematobium and S. mansoni in man. In subsequent clinical trials,[93-100] niridazole given orally in daily doses of 20-30 mg/kg for 5-10 days afforded "cure rates" of 33-100% against S. haematobium[93-95,98,100] and 23-91% against S. mansoni.[93,96,97,99,100] The drug has uncertain value against S. japonicum in man.[5] Minor drug reactions including headache, abdominal pain, nausea and vomiting, and ECG changes were frequently observed but were seldom severe enough to warrant cessation of therapy.[93-99] However, infrequent but serious toxic reactions, including epileptiform seizures, psychoses, and epistaxis, were also encountered.[83,97-99] Therefore, several investigators have recommended that niridazole be used only under strict medical supervision and that unsupervised mass treatment be approached with caution.[97-99]

Studies in mice with $^{14}C$ niridazole showed that unchanged drug is taken up from body fluids both by adult S. mansoni and by embryonated eggs, and that metabolites accumulate in organs of the parasite.[101] Rapid metabolic conversion by the parasite appears to be responsible for the ensuing tissue damage, possibly by causing overloading of a particular enzyme system which might be a nitro-reductase.[101] Other nitrothiazole compounds reportedly have schistosomicidal activity.[71,80,81,102]

(b) Nitrofurans – Furapromidium (F30066)(XIX) kills S. japonicum in vitro and has prophylactic effects in mice and curative action in rabbits and dogs.[5] The drug was active against S. japonicum in early human trials, but the cure rate was disappointing at the regimen employed. In a recent trial, furapromidium administered orally in doses of 50-100 mg/kg for 1-4.5 months enabled a cumulative negative rate of 81%.[103] The authors consider furapromidium to be a suitable alternative when antimonial treatment is contraindicated.[103] Other nitrofurans are reported to be active.[104,105]



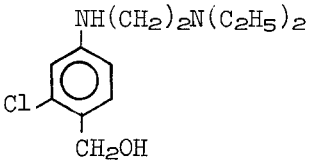
XIX Furapromidium



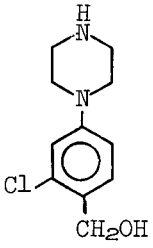
a, R=H (lucanthone)
b, R=OH (hycanthone)

XX

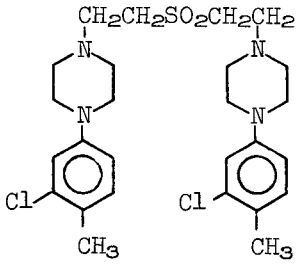
(c) Lucanthone Relatives – Hycanthone (XXb),[3,106] a new metabolite of lucanthone (XXa), was reported earlier to be much more potent than lucanthone against S. mansoni in hamsters.[106] However, in a recent comparison of the relative effectiveness of hycanthone against S. mansoni in various hosts, it was only one-tenth as active in mice as in hamsters.[107] The drug has pronounced activity against S. mansoni in Cebus monkeys,[107] but no reports have yet appeared on its efficacy and tolerance in man. Numerous other substances related to lucanthone, hycanthone, and mirasan exhibit antischisto-



XXI



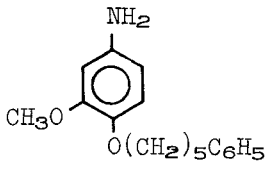
XXII



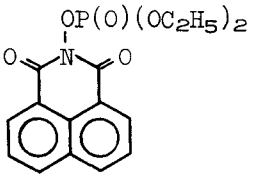
XXIII

some activity, among which compounds XXI-XXIII are representative.[108,109]

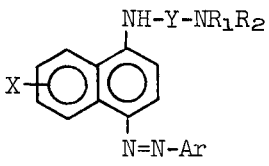
(d) Aminophenoxyalkanes – Preliminary clinical reports indicated that N-[5-(p-aminophenoxy)pentyl]phthalimide (amphotalide)[2,110] produces a definite therapeutic response in S. haematobium infections at fairly well-tolerated doses, but diminution of the visual field was observed in several patients. The search was therefore continued for compounds devoid of retinal toxicity and more active than amphotalide in experimental animals.[110,111] Among hundreds of aminophenoxyalkanes that were synthesized[111] and tested,[110] XXIV was selected for initial clinical trial. A total dose of 60-120 mg/kg over 1-4 days cured 16 of 44 subjects infected with S. haematobium. The drug gave rise to no ocular symptoms, but did produce serious vomiting.[110]



XXIV M and B 3838A



XXV Maretin



XXVI

(e) Organophosphorus Compounds — The motor responses of S. mansoni to various pharmacological agents suggest the presence in the worm of an inhibitory cholinergic system.[112] Carbachol, arecoline, and a variety of cholinesterase inhibitors produced a paralysis of the worms which was reversed by atropine and by secondary and tertiary amines, but not by quaternary ammonium ganglion blocking agents.[112]

Tartar emetic and the tris(p-aminophenyl)carbonium salts inhibit cholinesterase activity in schistosomes.[3] There is thus some rationale for the evaluation of potential cholinesterase inhibitors against schistosomiasis. Trichlorofon is reported to have activity against S. japonicum in mice, dogs, and man[113] and against S. haematobium in man.[5,95] However, tests against S. mansoni in mice and monkeys showed insignificant action by large oral doses of the drug,[113,114] and the concentration required to produce paralysis of S. mansoni in vitro is rather high when compared with the dosage claimed to be effective chemotherapeutically against S. haematobium and S. japonicum.[112] Therefore, it is questionable whether cholinesterase inhibition can account for the antischistosomal action of trichlorofon. It is perhaps too early to pass judgment on the clinical usefulness of trichlorofon, but its known toxicity for man discourages its use.[5]

In contrast with trichlorofon, maretin (XXV) and the corresponding thioate ester exhibited very potent oral activity against S. mansoni in mice.[113-114] However, neither compound showed strong activity against S. mansoni in monkeys at well-tolerated dose levels, and both failed to kill worms in vitro in concentrations of 16 μg/ml.[113] The lack of in vitro activity suggests either an insufficient drug concentration in the medium, or conversion to an active metabolite by the host.

(f) N,N-Dialkyl-N'-(4-arylazo-1-naphthyl)alkylenediamines (XXVI) exhibit widespread chemotherapeutic and prophylactic activity against S. mansoni in mice and monkeys and against S. japonicum in mice.[115,116] The usefulness of these compounds in human schistosomiasis has not been evaluated.

INTESTINAL HELMINTHIASES — The world-wide incidence of intestinal helminthic infections in man approaches the staggering figure of two billion.[2] The prevalence of these infections and their complex and far-reaching effects upon body function cause them to rank among the most important disease problems of man.

(a) Drugs Active Against Intestinal Nematodes

(1) Thiabendazole — Reports on the value of thiabendazole against human

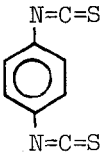
helminthiases were summarized recently.[5,117] The drug has broad action, particularly in multiple doses, in suppressing egg production by most of the important intestinal nematodes of man, but is relatively ineffective against Trichuris. Effective doses frequently cause dizziness, and to a lesser extent nausea and vomiting. Relative to other anthelmintics, thiabendazole is likely to excel in the treatment of strongyloidiasis, creeping eruption, and possibly trichinosis.[5]

(2) Pyrvinium pamoate[2] has been used mainly in the single dose treatment of enterobiasis, but more recent evidence indicates it is also useful against strongyloidiasis when given over a period of 1-2 weeks.[5] CI-578, a combination drug containing pyrvinium pamoate and piperazine, was developed to incorporate the efficacy of pyrvinium pamoate against Enterobius vermicularis and piperazine against Ascaris lumbricoides into a single convenient medication. In preliminary clinical trials, CI-578 showed marked activity against single or multiple pinworm and ascarid infections when given in a single oral dose (2.5 mg of pyrvinium base and 75 mg of piperazine per kg of body weight) on 2 successive days.[118,119] Among 71 subjects treated, the cure rates for E. vermicularis and A. lumbricoides were 98% and 68%, respectively; a significant reduction in egg counts was noted in the remaining patients. The drug was well tolerated.[118,119]

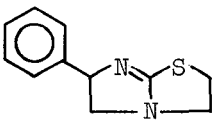
(3) Dymanthine hydrochloride (N,N-dimethyloctadecylamine hydrochloride) is active against Syphacia obvelata and Hymenolepis nana in mice. In a preliminary clinical trial, 32 of 50 subjects (64%) infected with Ancylostoma duodenale were cured following a single oral 50 mg/kg dose of dymanthine hydrochloride.[120] Gastrointestinal side-effects were frequently observed but were mild and amenable to symptomatic treatment.[120]

(4) 1,4-Phenylenediisothiocyanate (XXVII) was reported earlier to be effective against nematodes and cestodes in experimental animals. Subsequently, the drug was administered to 43 patients with hookworm disease in a dose of 300 mg every 12 hr.[121] Among 38 patients followed-up, 21 (55%) were cured and 13 others showed a substantial reduction in egg count. Mild gastrointestinal side-effects were noted in 17 subjects.[121]

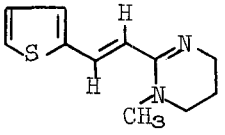
(5) Other Anthelmintics — Efforts directed primarily toward the development of anthelmintics for veterinary use (Chapter 14b) have recently yielded a variety of active nematocides of potential usefulness in man.[3] Among them, various benzimidazoles,[76,122-124] tetramisole (XXVIII) and relatives,[125]



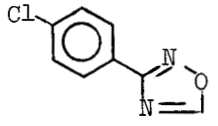

XXVII




XXVIII Tetramisole




XXIX Pyrantel



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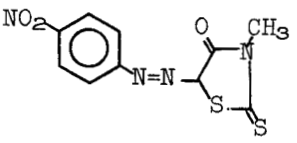
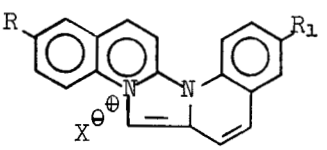


XXXI Nitrodan



XXXII

pyrantel (XXIX)[126] and analogs,[127] 1,2,4-oxadiazoles (XXX),[128] nitrodan (XXXI),[129,130] and imidazodiquinolinium salts (XXXII)[131] are noteworthy.

(b) Drugs Active Against Cestodes

Paromomycin sulfate is a basic oligosaccharide-type antibiotic with unique action against Entamoeba and Trichomonas.[2,5] It has recently been reported to be highly effective against the human tapeworms Taenia saginata,[132-134] Taenia solium,[133] and Hymenolepis nana.[133] Doses of 15 to 45 mg/kg daily for 3-5 days were most frequently employed, although larger single doses were also effective.[134] Subsequent studies in animals showed that paromomycin had inconsequential effect against H. nana in mice or H. diminuta in in rats, but strong action against Hydatigera taeniaeformis in vitro and in cats.[135] Although the mode of action of paromomycin against parasites is unknown, it inhibits the incorporation of amino acids into the trypsin-soluble protein fraction of Staphylococcus aureus 257.[136] At the ribosomal level, paromomycin prevents the attachment of amino acyl-s-RNA and causes accumulation of m-RNA.[136]

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# Chapter 14(b). Animal Antiparasitic Agents

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## Anthelmintics for Treatment of Roundworm Infections

Pyrantel tartrate, representing a new class of anthelmintics, was announced in 1966. The therapeutic properties of tetramisole, reported in the previous year, were confirmed in a series of papers describing laboratory and field studies. The data presently available in the published literature are not yet sufficient to allow a detailed comparison of these new anthelmintics and thiabendazole, but some important similarities and differences can now be discerned. All three are broad-spectrum and are efficacious against many species of important nematodes parasitizing the entire gastrointestinal tract of cattle and sheep. Poor control of Trichuris is a weakness common to all three. An impressive breadth of spectrum for tetramisole was suggested in the preliminary announcement of this drug, in which activity against at least 56 species of nematodes in thirteen hosts was listed.[1]

Pyrantel tartrate has a somewhat higher safety factor than tetramisole, according to published accounts (see Table I), though neither approaches the high therapeutic index reported for thiabendazole.

Differences in the effects of these three anthelmintics against lungworm infections may be due to the varied physiological dispositions of these drugs, reflecting differences in absorption and metabolism. Tetramisole was reported to be effective against lungworms. Thiabendazole, which is rapidly metabolized, has been shown to have a significant effect against lungworms (vide infra), although special dosing schedules are required. Pyrantel tartrate, not being well absorbed from the intestine, was said to be inactive against lungworms.[2]

A comparison of the relative potencies (in terms of recommended oral doses for control of parasitic gastroenteritis in sheep), minimum lethal dose (sheep), and the safety factor for these three substances is given in the table:

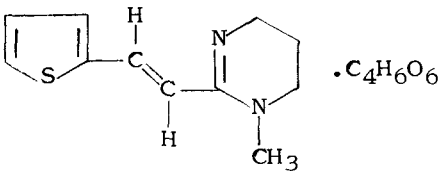
TABLE I

| Compound | Effective Dose | MLD | Safety Factor |
|---|---|---|---|
| Pyrantel Tartrate | 25 mg/kg | 180 mg/kg[3] | ca. 7[3] |
| Tetramisole | 15 mg/kg | 80 mg/kg[4] | ca. 4-5[4] |
| Thiabendazole | 44 mg/kg | >1000 mg/kg[4a] | > 23[4a] |

## Pyrantel Tartrate

This substance, which has the structure trans-1-methyl-2-[2-(2-thienyl)vinyl]-1,4,5,6-tetrahydropyrimidine (I), was the most promising member of a new class of anthelmintics announced by W. C. Austin and coworkers.[5] These compounds evolved from laboratory assays employing Nematospiroides dubius in mice and Nippostrongylus muris in rats. From a brief discussion of structure-activity relationships in the series, it appears that a lower order of anthelmintic potency was found with relatives in which the intercyclic vinylene linkage was fully saturated or in which the tetrahydropyrimidine ring was replaced with an imidazoline. Methylation of the secondary ring nitrogen was beneficial, and introduction of a methyl substituent into the 3-position of the thiophene ring apparently leads to enhanced potency.

Description of chemical procedures for preparation of pyrantel salts and related structures appeared in a series of Pfizer patents.[6-9]




$\cdot C_4H_6O_6$

Pyrantel Tartrate
I

Pyrantel tartrate was described as a colorless crystalline material which is soluble in water (180 mg/ml). Solutions are sensitive to ultraviolet light, undergoing trans-cis isomerization upon prolonged exposure. Storage in dark bottles is sufficient to protect solutions against light-induced degradation.[3]

In laboratory trials, the substance, administered orally as a 5% solution in water, gave good control of Trichostrongylus colubriformis infections of sheep at 45 mg/kg or 25 mg/kg.[3] Doses of 12.5 or 25 mg/kg resulted in substantially complete elimination of Nematodirus battus from experimentally infected lambs. In a trial with naturally infected sheep, pyrantel tartrate at 25 mg/kg, orally, was effective in the removal of Ostertagia, Trichostrongylus, Nematodirus, Cooperia, and Chabertia, but had little effect on Trichuris.[3] Death of one lamb out of six was seen following use of an oral dose of 180 mg/kg. 100 mg/kg was well tolerated in twelve sheep.

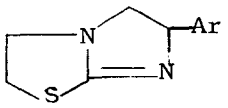
Efficacy of pyrantel for the prevention of parasitic gastroenteritis in sheep was demonstrated in field trials with lamb flocks harboring variable but moderate infections of Nematodirus, Ostertagia, Trichostrongylus, and Haemonchus.[10,11] Under the conditions of these trials, pyrantel was found to result in somewhat greater average daily weight gains than the other anthelmintics used for comparison (bephenium, methyridine, thiabendazole), though the objection was made later that the thiabendazole dose used in the Nematodirus trials was

less than the dose recommended by the manufacturer for control of Nematodiriasis.[12]

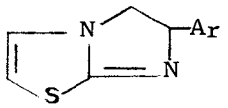
Reference was also made to good activity with pyrantel against Ascaris in swine and against Toxocara and Toxascaris in dogs.[5]

## Tetramisole

The circumstances which led to the discovery of tetramisole (II, Ar = $C_6H_5$), a new broad-spectrum anthelmintic, were described in two papers from the Janssen Laboratories.[1,13] 2-Acetylimino-3-[2-hydroxy-2-(2-thienyl)ethyl]thiazoline (Thiazothienol, IV), which was under investigation as an anthelmintic in poultry and sheep, was found to undergo metabolism in these species to the more potent 6-(2-thienyl)-5,6-dihydroimidazo[2,1-b]thiazole (III, Ar = 2-thienyl). This metabolic transformation was not observed in rats or mice.



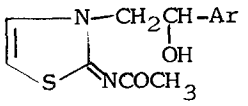

II




III

Tetramisole, Ar = $C_6H_5$

Tetramisole was the product of systematic development of reduced imidazo[2,1-b]thiazoles of the types II and III. Thirty-three examples of both types were described,[13] but detailed structure-activity relationships were not discussed.




IV

A more detailed report of the metabolic studies with thiazothienol appeared in a new paper by Allewijn and Demoen.[14]

Walley[4] examined the action of tetramisole against natural and experimental infections with parasitic nematodes in 1076 sheep and 18 goats. Oral doses of 15 mg/kg gave good results against adult and immature roundworm species in the abomasum and intestines, with the exception of Trichuris. The same dose, or 10 mg/kg, was effective against the lungworm Dictyocaulus filaria in these species. At 10 mg/kg, the drug resulted in 82-94% removal of immature lungworms seven days post-infection, and 41% three days after infection (sheep).

Slight side-effects were observed with doses of 15-20 mg/kg orally[4]; the animals showing depression or occasional twitches. Convulsions followed administration of 50 mg/kg doses, but the animals recovered. One of twenty sheep dosed with 80 mg/kg died.

Efficacy of tetramisole against a broad spectrum of gastrointestinal parasites and lungworms in sheep and goats was confirmed in a series of field trials by several investigators.[15-21]

Ross[16] found that tetramisole at 12.5 or 15 mg/kg, orally, was effective against 3-day-old or adult Ostertagia circumcincta, but failed to remove a high proportion of the parasites when given ten

days after infection.

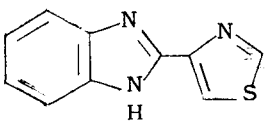
Fitzsimmons[21] noted erratic action of tetramisole (12.5 or 25 mg/kg, p.o.) against 3rd and 4th-stage larvae of Trichostrongylus colubriformis in goats, though the agent was highly effective against the adults of this species at both levels.

Guilhon[20] recorded incomplete removal of Bunostomum trigonocephalum even at 20 mg/kg sub-cutaneously (sheep). Walley[4], on the other hand, showed good control of Bunostomum spp. at 10 mg/kg, s.c., in two sheep, and generally good control of these species with the standard oral doses, although the results were somewhat erratic.

Guilhon[20] observed deaths of sheep using 50 mg/kg of tetramisole, sub-cutaneously. Walley[4] suggested that 50 mg/kg is the toxic dose for parenteral tetramisole, but noted that the compound is more effective by this route, hence the therapeutic index should not be substantially altered.

Preliminary accounts of trials with tetramisole in cattle have appeared.[22,23] A subcutaneous dose of 10 mg/kg gave good results against adult and immature Dictyocaulus viviparus, Ostertagia, and Cooperia, except for poor control of fifth-stage larvae of Ostertagia. Nearly all calves receiving this treatment showed moderate signs of toxicity, and tissue reactions were observed at the injection site in about half of the animals.

Thiabendazole



V

Thiabendazole (V) was found effective against larval and adult lungworms of cattle, Dictyocaulus viviparus, but only under specialized conditions of administration.[24] Use of three consecutive daily intramuscular injections of 33-110 mg/kg resulted in 100% clearance of immature parasites (15-17 days post-infection). Three daily treatments with 44 mg/kg of thiabendazole, intramuscularly, removed 95.5% of adult D. viviparus. Oral, intratracheal, or intraperitoneal routes were ineffective or unsatisfactory.[24,25,26] The lack of effectiveness of single dose treatments with thiabendazole for the treatment of lungworms was attributed to the known[27,28] rapid conversion of this substance to inactive metabolites. Irritation and residues at the injection site limit the usefulness of presently available dosage forms of thiabendazole for intramuscular treatment of lungworm infections.

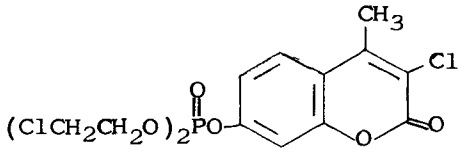
The rat lungworm, Angiostrongylus cantonensis, was removed by 200 mg/kg doses of thiabendazole.[29]

Thiabendazole was reported effective against mature Oesophagostomum spp. in swine at a rate of 66 mg/kg (p.o.), but to be

without effect against the tissue-phase parasites at 5 days after infection.[30] The effects of thiabendazole on therapy and prophylaxis of Trichinella spiralis in swine were described by Campbell and Cuckler.[31]

## Other Nematocides

A new installment in the interesting story of the selective effect of haloxon (VI) on helminth cholinesterase appeared recently.[32] It had been shown earlier that the phosphorylated enzyme; i.e., bis-(dichloroethoxy)-phosphoryl cholinesterase, which arises from interaction of haloxon with a cholinesterase of sheep, dephosphorylates rapidly compared to the corresponding phosphorylated enzyme which formed from haloxon and cholinesterase from Haemonchus contortus.[33,34] The higher therapeutic index of haloxon relative to older organophosphate anthelmintics was attributed to this observed biochemical difference between host and parasite.



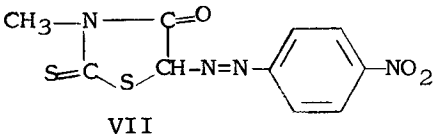
Haloxon

VI

The study has now been extended to include parasitic nematodes other than H. contortus. It was found that three other species which are sensitive to haloxon; namely Oesophagostomum venulosum, O. dentatum, and Ascaris lumbricoides, also contain a cholinesterase which is efficiently deactivated by haloxon. The original hypothesis for the basis of selectivity observed with haloxon was supported by the observation that phosphorylated enzymes from Dictyocaulus filaria, a species which is not sensitive to haloxon and from Bunostomum trigonocephalum, which has only fair susceptibility to haloxon, are rapidly reactivated, presumably via ready dephosphorylation. Three nematode species which are normally located in the large intestine gave quite different results. Though infections with these species do not respond readily to treatment with haloxon, cholinesterase from the parasites was rapidly deactivated by the drug. Failure of the correlation with these helminths was attributed to the possibility that their physical location protects them from lethal concentrations of the anthelmintic.

The correlations between specific anticholinesterase inhibition and anthelmintic action of haloxon is not perfect, but they help to explain the unusual properties of this drug. Perhaps the information derived from these studies will lead to discovery of new anthelmintic organophosphates with even higher selectivity of action between nematodes and mammals.

3-Methyl-5-(p-nitrophenylazo)rhodanine (Nitrodan®, VII) is an interesting anthelmintic with quite a different spectrum of action from the nematocides discussed above.



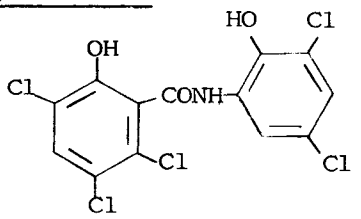
VII

When administered continuously in the diet of experimental animals, it brought about the removal of the pinworm Syphacia obvelata in mice, Ascaridia galli in chickens, and Toxocara canis, Ancylostoma caninum, and Uncinaria stenocephala in dogs. The effective dose levels were dependent upon duration of treatment and ranged from 0.016% to 0.1% in the diet.[35]

The drug was ineffective against Nippostrongylus muris, Aspicularis tetraptera, or Nematospiroides dubius in mice and Toxascaris leonina in dogs.

## Anthelmintics for the Treatment of Liver Fluke Infections

### Oxyclozanide



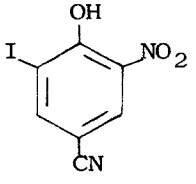
Oxyclozanide

VIII

The utility of oxyclozanide (VIII) for treatment of adult liver fluke infections in cattle and sheep was demonstrated in laboratory tests[36,37] and several field trials.[38-40] It was effective against adult flukes in sheep at 15 mg/kg, and in cattle at 10-15 mg/kg. Toxic side-effects were observed in doses above 30 mg/kg, with deaths occurring at 60 mg/kg (sheep and cattle). The safety factor was considered to be four in average animals, but less in those which are dehydrated or suffering from extensive liver damage. Like the other fasciolacides now available[37], oxyclozanide has an effect against immature flukes (6 weeks or older), but only at higher and toxic dose levels.[36,37,39]

### M&B 10,755

Preliminary accounts of a newer modification of the nitrophenol class of anthelmintics appeared.[41,42] This compound, M&B 10,755 (IX), was reported active at 8 mg/kg in sheep or calves (1/5 of the maximum tolerated dose) against mature Fasciola hepatica, when given intramuscularly or subcutaneously. Activity against immature (4-6 weeks) flukes was observed at the higher dose of 20 mg/kg (1/2 of the MTD). Oral dosing was less satisfactory.



M&B 10,755 IX

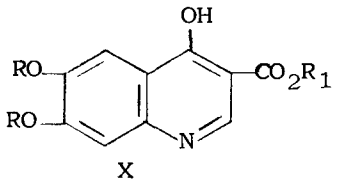
A paper by Boray, Happich, and Andrews[37] contains a comparative study of the liver fluke agents carbon tetrachloride, hexachloroethane, hetol (1,4-bis-trichloromethylbenzene), hexachlorophene, hexachlorophene monophosphate, Hilomid ® (a combination of

3,5,4'-tribromosalicylanilide and 5,4'-dibromosalicylanilide), 2,2'-dihydroxy-3,3'-dichloro-5,5'-dinitrobiphenyl, oxyclozanide, and disophenol (2,6-diiodo-4-nitrophenol). None of these substances was effective against 4- or 6-week-old infections at the doses which are adequate for eradication of mature flukes. Removal of these immature parasites could be demonstrated with all of the compounds tested, but in every case only at toxic levels. The authors emphasized the importance of anthelmintics effective against immature infections, particularly for controlling acute outbreaks of fascioliasis. There exists a clear need for new fasciolacides which are capable of eradicating immature liver flukes at safe doses.

## Coccidiosis

### Buquinolate

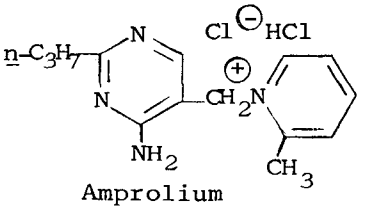
Many examples of a variety of alkyl 4-hydroxy-6,7-dialkoxyquinoline-3-carboxylates (X) were found to possess a high order of coccidiostatic activity when tested for prevention of experimental Eimeria tenella infections in chicks.[43] The preferred examples were ethyl esters, but relative potency was most sensitive to the nature of the alkoxy groups in the 6- and 7-positions of the quinoline ring. $R_1$ was varied from methyl to propyl, isopropyl, and allyl. Similarly, homologous series wherein the groups R and $C_1$ to $C_{10}$ straight- and branched-chain hydrocarbon residues were examined. Highest potency was obtained with 6,7-bis-(isobutoxy)quinolines, and ethyl 4-hydroxy-6,7-bis-(isobutoxy)-quinoline-3-carboxylate [X, $R_1 = C_2H_5$, R = $(CH_3)_2CHCH_2$] was selected for extensive evaluation and was given the generic name buquinolate.



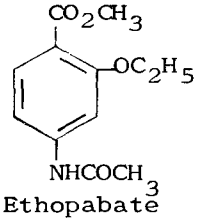
X

Buquinolate, R = $(CH_3)_2CHCH_2$; $R_1 = C_2H_5$.

In laboratory and pen trials, buquinolate at 0.00825% in the diet was effective for protection of chicks from mortality and morbidity due to exposure to nine species of Eimeria.[43-47] A dietary level of 0.00825% of buquinolate was judged similar in coccidiostatic efficacy to a combination of amprolium (XI) (0.0125%) and ethopabate (XII) (0.0004%).[46]



Amprolium
XI



Ethopabate
XII

Buquinolate was found to be poorly absorbed from the chicken intestine[48], and to possess low acute and sub-acute toxicities.[49] The maximum tolerated single oral doses were reported to be in excess of 20 g/kg and 10 g/kg

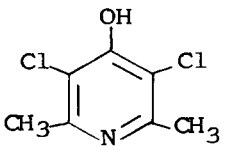
in chickens and turkeys, respectively, and greater than 7.5 g/kg in mice and rats. Chickens tolerated continuous feeding of a diet concentration of 0.088% for 31 days or 0.044% for ten weeks without signs of toxicity. Use of up to three times the recommended dose in chickens had no adverse effect on growth.[44-45,50-53] The recommended dietary level of 0.00825% was said to have no harmful effect on feed conversion, egg production, egg quality, hatchability, or fertility.[51,52,53] Lower efficiency against turkey coccidiosis was suggested in one report.[44]

The coccidiostatic nature of buquinolate was demonstrated by resumption of development of E. necatrix infections which could be observed seven days following withdrawal of the drug.[54].

Quinolines related in structure to buquinolate were described as coccidiostats in a recent patent from Imperial Chemical Industries.[55]

## Meticlorpindol

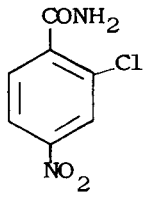
In preliminary accounts, 2,6-dimethyl-3,5-dichloro-4-hydroxypyridine (meticlorpindol, XIII), was reported to afford good protection against nine species of coccidia in chicks when fed in the diet at 0.0125%.[56,57] Weight gains and feed efficiency in birds treated with meticlorpindol were judged good.



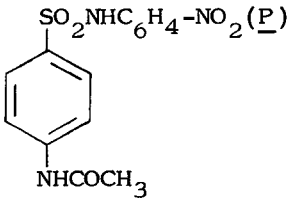
Meticlorpindol
XIII

## Novastat®

Brown, Mueller, and Morehouse[58] reported studies with 2-chloro-4-nitrobenzamide (XIV) and combinations of XIV with N-(p-nitrophenyl)-4-acetamidobenzenesulfonamide (XV) against coccidiosis. These authors noted that tolerated levels of XIV were able to protect chickens from mortality due to exposure to E. necatrix or E. tenella, but that protection against E. acervulina could be achieved only at toxic levels with XIV alone. A combination equivalent to a final dietary concentration of 0.025% of XIV and 0.020% of XV, designated Novastat®, prevented development of coccidiosis when tested against all three Eimeria species E. tenella, E. necatrix, and E. acervulina. Use of Novastat® resulted in no reduction of growth response in chicks, and a 3.9% improvement in feed efficiency, relative to uninfected, unmedicated controls.



XIV



XV

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# Chapter 15. Antifungal Agents

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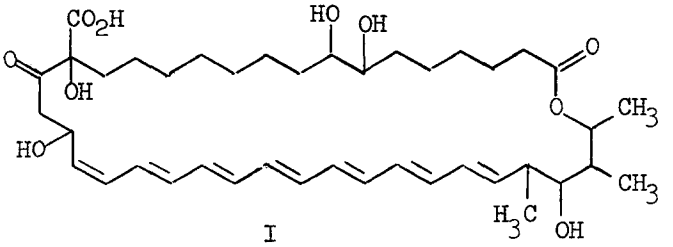
The many publications in this area during 1966 have covered both the elaboration and verification of the usefulness of previously known drugs and the announcement of new compounds with potentially interesting activity. Considerable effort has also been given to the study of the mode of action of various antifungal antibiotics. The material in this chapter is divided primarily into sections on polyene antibiotics, non-polyene antibiotics and synthetic compounds.

Polyene Antibiotics--This class of compounds is of considerable usefulness, primarily for the treatment of systemic mycoses and conditions caused by various species of Candida.

Clinical reports on the curative effect of amphotericin B continue to appear. They include its successful use in the following systemic fungal diseases: blastomycosis,[1,2,3] coccidioidomycoses,[4,5] cryptococcosis,[6,7] Candida endocarditis,[8] Candida meningitis,[9] meningitis due to Sporotrichum schenkii,[10] and histoplasmosis.[11,12] A new therapeutic approach involving adjustment of the dosage of this antibiotic with regard to the patient's renal function and the serum level of the drug has allowed the use of smaller doses than those generally recommended.[13] The complete structure of amphotericin B is still unsolved. However, one report[14] suggests that a partial structure for amphotericin B aglycone can be formulated as I. Further work is necessary to determine the complete oxygenation pattern and the position of attachment of the mycosamine moiety to the aglycone. A second report suggests a similar structure in less detail.[15] A timely review article[16] covers toxicity, mechanism of action and therapeutic usefulness of amphotericin B.

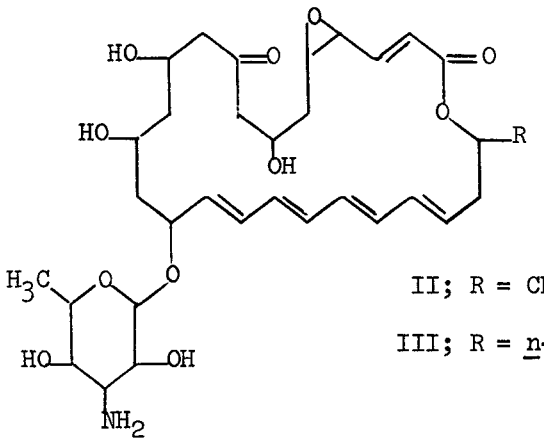


I

Nystatin has been found to be a satisfactory drug for the treatment of vaginal moniliasis in nonpregnant women[17] while in oral candidiasis its sodium salt in the form of an aerosol was reported to be efficacious.[18]

A clinical trial involving 355 patients with various skin ailments has delineated the usefulness of a lotion containing pimaricin, neomycin

and hydrocortisone. A general cure-rate of 70% was seen.[19] Several other clinical trials have shown pimaricin to be useful particularly in vaginal moniliasis[20,21,22] and a review article has appeared.[23] A corrected structure for pimaricin (II) has been reported[24] while lucensomycin (III) was found to be a homolog.[25]




II; R = $CH_3$

III; R = n-$CH_2CH_2CH_2CH_3$

The relatively new antibiotic, candicidin, has given excellent results in the treatment of vaginal moniliasis[26] and fair results with cutaneous moniliasis[27] but good comparative studies with previously known antifungal agents have not been reported.[28]

Hamycin, used for the treatment of 30 patients with proven vaginal moniliasis, produced cures in 29 patients and was considered to be an outstanding drug for the treatment of this disease.[29] In contrast to this its use in 10 cases of systemic mycoses resulted in improvement in only two cases.[30] A question has been raised as to the possible identity, or at least close similarity of hamycin, trichomycin and candicidin.[31]

The mode of action of polyene antibiotics has been the subject of a number of reports in 1966 among which are three review articles[32,33,34] The general theory is that these antibiotics cause a change in the permeability of the fungal cell membrane with attendant depletion of essential cellular constituents and that this change in permeability is in some way triggered by a binding of the antibiotic to the cell membrane via a steroid-antibiotic complex. Further support for this theory, which in effect says that the presence of a steroid in the cell membrane is indeed a necessary prerequisite for polyene sensitivity, came as the result of an in vitro examination of the effects of polyene antibiotics on model bilayer membrane systems of egg lecithin, with and without cholesterol.[35]

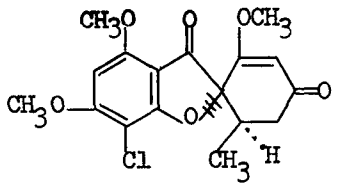
Earlier studies had shown that added steroids could prevent the antifungal activity of some polyene antibiotics possibly by complexing with the antibiotic. A more recent study[36] using Candida albicans has now shown that wide differences exist among these antibiotics with reference to the variety of added steroids which will interfere with antifungal

activity. In addition a similar variation exists in the ability of various antibiotics to initiate rapid loss of potassium ion.[37]

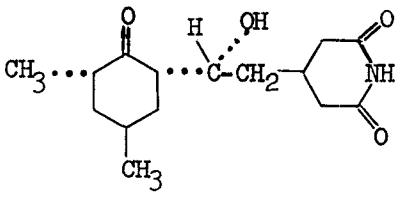
Nonpolyene Antibiotics--The mode of action of griseofulvin (IV), a subject relatively neglected in the past, is now receiving attention. It has been suggested[38] that this antibiotic interferes with the replication mechanism of fungal cells. However, a review article[39] indicates that despite the rather large amount of recent work there is at present no clear answer to this question. Several studies on the metabolism of griseofulvin show that in the rat, two major metabolites, 6- and 4-demethylgriseofulvin, are produced,[40,41] while in the rabbit the only primary metabolite is the 6-demethyl derivative.[41] p-Ethoxyacetanilide and p-methoxybenzylamine were found to inhibit this metabolism in rat liver slices.[42] An investigation of the origin of O-methyl groups in griseofulvin using $^{14}C$-labelled substrates revealed that pyruvic acid, serine, and formic acid supplied carbon to all three methoxyl groups.[43]

An evaluation of microcrystalline griseofulvin therapy (oral) in scalp ringworm using 324 patients emphasizes that successful therapy requires different treatment schedules for nonfluorescent Trichophyton induced infections and those caused by Microsporum species.[44] Although griseofulvin (IV) is usually given orally (as above) a recent report[45] discloses successful topical treatment of 29 cases of superficial dermatophytosis with a 5% griseofulvin ointment.

A ring-B carbon analog of griseofulvin was synthesized but had no significant antifungal activity.[46]



IV

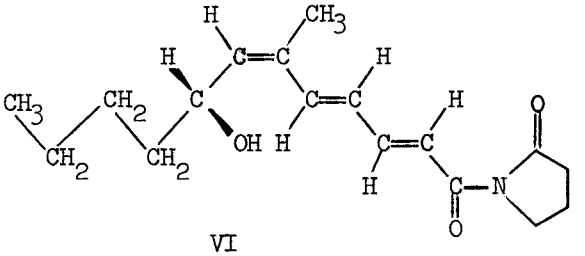


V

A structure-activity study of cycloheximide (V), its derivatives and analogs using Saccharomyces pastorianus suggested that keto, hydroxyl, and imide nitrogen groups may be involved in a 3-point attachment of antibiotic to the sight of action.[47] The inhibitory effects of cycloheximide in yeast, algae, and mammalian cells seem to resemble one another in that in each case the primary effect is an inhibition of protein synthesis, whereas inhibition of nucleic acid synthesis is indirect.[48]

The polypeptide antifungal agent, X-5079C, used in 39 patients, was active against North American blastomycosis, histoplasmosis, and sporotrichosis[49,49a] but under the drug regimen that was used the relapse rate was rather high.[49]

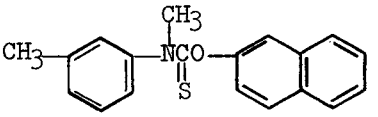
Variotin, for which a detailed structure (VI) has now been reported,[50] has been used in the form of an ointment to treat 72 patients with ringworm infections caused primarily by T. rubrum and T. mentagrophytes. The results, 43 cured and 17 with improved conditions, suggest that further trials are warranted.[51]



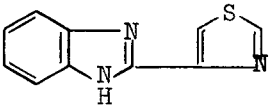
VI

Preliminary data indicate that the following antibiotics may be potentially useful: pyrrolnitrin,[52] C-1 from C. vincetoxicum,[53] necrotoxin,[54] sporaviridin,[55] ophiobolosin,[56] frenolicin,[57] and rufochromomycin.[58]

Synthetic Antifungal Agents--Probably the most interesting of the antifungal agents developed during the last few years is tolnaftate (VII). Clinical trials reported during 1966 have continued to show that this compound is a highly effective and extremely well tolerated[59] specific agent in the treatment of superficial mycoses caused by dermatophytes.[60,61,62,63] It is noteworthy, however, that two cases of tinea nigra caused by Cladosporium wernecki were not cured.[64] A teratogenesis study indicated that tolnaftate does not adversely affect rat or mouse fetal and postnatal development.[65]

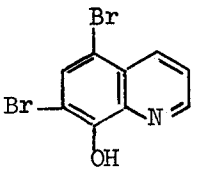


VII

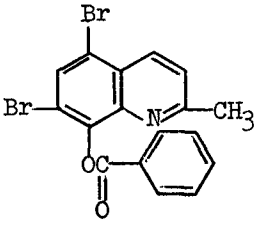


VIII

Several clinical studies[66,67,68] with thiabendazole (VIII), a broad-spectrum systemic anthelmintic, have shown it to be potentially useful in superficial fungus infections of the skin, particularly dermatophytic infections of the groin[66] and feet.[67] Cutaneous moniliasis is not affected.[66] A structure-activity study on sixty-eight 8-hydroxyquinolines[69] led to the selection of 5,7-dichloro-8-nicotinoyloxyquinoline as the most active compound. The latter compound was tested in 24 patients with mycoses caused by trichophyton species. Total recovery of 14 patients and marked improvement in 4 was reported.[69] An ointment containing broxyquinoline (IX) and brobenzoxalidine (X) ( an intestinal antiseptic and fungicide) showed promise in the treatment of superficial mycoses of the skin.[70] Topical treatment of 398 patients gave improvement in 82% of the cases as follows: after 7 - 10 days 119 had cleared and after 11 - 21 days another 209 had cleared.



IX



X

A regimen of treatment for ringworm of the nails which included the use of a 30% pyrogallol plaster, 1% tetramethylthiuram disulfide and 1% dimethylaurylbenzylammonium chloride appears to have been highly successful. In 50 patients with 283 infected nails 96% of the finger nails and 91% of the toe nails were cured.[71] The relapse or reinfection rate was 12-14%. A rapid, single, overnight treatment of tinea versicolor with a 2.5% selenium sulfide suspension appears to be useful despite a rather high recurrence rate. Out of 98 patients 81 showed complete elimination of the fungus while another 9 showed marked improvement.[72]

Although not generally appreciated polynoxylin (polyoxymethyleneurea) appears to be useful in the treatment of oral candidiasis. Of twenty-four cases treated with polynoxylin lozenges for one month 17 were cured and the other 7 showed improvement.[73] Among a group of 200 cases of vaginal infection of various kinds treated with a preparation containing 1% 0-iodobenzoic acid and 11% triethanolamine 15 patients with vaginal monoliasis gave results rated as good to excellent in 95% of the cases.[74] The treatment of 150 cases of monilial skin infections with a cream containing 0.1% penotrane (phenylmercury 2,2'-dinaphthylmethane-3,3'-disulfonate) and 0.25% prednisolone resulted in clearing of the infection in 75% of the cases and improvement in 15%.[75] No sensitivity reactions were observed.

Subcutaneous phycomycosis, a tumor associated with the fungus Basidiobolus haposporus or B. meristosporus, appears to be susceptible to treatment with potassium iodide. Of 12 cases seen 5 of them could be treated regularly with potassium iodide and all of these recovered.[76] Previous attempts to treat other cases with nystatin or griseofulvin had been unsuccessful.

11-Iodo-10-undecylenic acid is claimed to be superior to 10-undecylenic acid for the treatment of a dermatophytosis of the skin of guinea pigs caused by trichophyton species.[77] The iodo derivative in a 1% solution gave an 82% cure while undecylenic acid in a 10% solution gave only a 23% cure.

An *in vitro* structure-activity study of 34 simple derivatives of 4-thiocyanatoaniline was carried out using 8 dermatophytes (3 genera). One compound, 2,6-dichloro-4-thiocyanatoaniline, was found to be active topi-

cally in guinea pigs but in man it produced skin sensitization.[78] The authors comment on the inadequacy of animal tests to detect compounds causing sensitization reactions in man.

In Vitro Synthetics--Many compounds are regularly claimed, with varying degrees of documentation to have in vitro antifungal activity. The following compounds and groups of compounds were selected as of interest on the basis of the degree, variety and reliability of documented activity: basically-substituted ethers of benzothiazole,[79] aryl- and aralkylthiocyanates and isothiocyanates,[80] 1,3-benzoxathioles,[81] 1,2-benzisothiazolones,[82] N-dichloroiodosalicylanilides,[83] 2,4-bis(arylamino) pyrimidines,[84] dehydroemetine,[85] 3,3'-dihydroxy-α,β-diethylstilbene,[86] 1,3-bis(2-chloroethyl)-1-nitrosourea,[87] nitrofuran derivatives,[88,89,90] 2-bromo-3-phenyl-2-propenal,[91] 2-hydroxythiobenzamides,[92] 1,3,5-thiadiazin-2-thiones,[93] carbonyl- and thiocarbamoyldisulfides,[94] cinnamylidene derivatives of α-aminohydroxamic acids,[95] amine oxides,[96] quaternary salts of basic esters of β(-1-naphthyl)acrylic acid,[97] 4-(2-aroylethyl)-1-piperazinecarboxylic acid esters,[98] N(t-aminoalkyl)-2-imidazolidones,[99] calcium capryl-2 lactylate[100] and esters of dithiocarbamic acids.[101]

Test Method--A technique has been described for the in vitro screening of large numbers of compounds for antifungal and/or antibacterial properties.[102] It involves the use of small tubes containing only 0.5 ml of medium and has been checked out with known antifungal agents. Its advantages as a screening procedure are described.[102]

Several general reviews have appeared within the last two years.[103,104,105]

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# Chapter 16 Antineoplastic Agents

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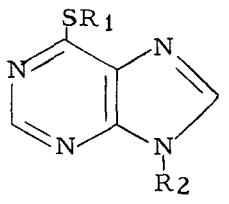
Introduction - Research on antineoplastic agents follows an orderly sequence. Drugs which exhibit antitumor activity in animals bearing transplanted neoplasms or cytotoxicity in cell cultures are considered for further study. These chemicals are examined with respect to toxicology, effects upon resistant tumors, and biochemical mechanism of action. The animal tumor screen continues in use in spite of widespread doubt as to its efficacy because no better method to select drugs for clinical trial against cancer has been found. Recently, Goldin, et al. have reviewed the results obtained in the screening system of the Cancer Chemotherapy National Service Center and suggested potentially useful simplifications;[1] Freireich, et al. have examined the predictive utility of preclinical toxicology with respect to anticancer agents;[2] and Zubrod and coworkers have analyzed the chemotherapy program of the National Cancer Institute.[3] Drugs which show consistent antitumor activity in pre-clinical studies are evaluated for therapeutic effects against various forms of cancer in man.

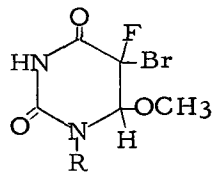
Purine and Pyrimidine Analogues - The following comments will center upon work published in 1966; Heidelberger has included earlier work in this area in his recent review.[4] Modifications of 6-mercaptopurine (I) are of continuing interest because of mercaptopurine's activity in human leukemia, and the ultimate development of resistance by the leukemic cells to this drug. 6-methylmercaptopurine riboside (II) is converted to 6-methylmercaptopurine ribonucleotide (III) by a nucleoside kinase. Cells that have lost inosinic pyrophosphorylase, a mechanism whereby they became resistant to I, are still inhibited by II. Bennett has demonstrated the presence of III in cells exposed to I and suggested that part of inhibitory effect of I may be mediated in part through its conversion to III;[5] a synergism between the antitumor effects of I and II has been reported in mice bearing Ehrlich ascites tumor.[6]




| | $R_1$ | $R_2$ |
|---|---|---|
| I | H | H |
| II | $CH_3$ | ribose |
| III | $CH_3$ | ribose-$PO_4$ |

The limited therapeutic activity of the fluorinated pyrimidines 5-fluorouracil (FU) and 5-fluoro-2'-deoxyuridine (FUdR) in certain forms of adenocarcinoma, principally of the digestive tract, have led to the synthesis of a variety of analogues. Dinucleoside phosphates containing FU

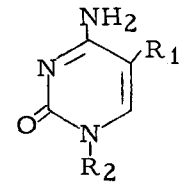
residues have been produced in the hope that they would be cleaved intracellularly thus generating the corresponding ribo- or deoxyribonucleotide without recourse to a nucleoside kinase reaction.[7,8] The concept is pertinent since loss, or lack, of enzymes in the pyrimidine salvage pathway is one potential mechanism of tumor resistance to FU or FUdR. Since β-5-fluoro-2-deoxyuridylyl-(5'-5')-β-5-fluorodeoxyuridine did not inhibit growth of Ehrlich ascites tumor cells resistant to FUdR,[7] the aforementioned hope has not been realized. A series of 5-halo-6-oxydihydro derivatives of the fluorinated pyrimidines has proven resistant to enzymatic degradation in animals; by spontaneous regeneration of the 5-6 double bond, FU or FUdR is released over a prolonged period of time.[9] One of these analogues, 5-bromo-6-methoxydihydro-5-fluoro-2'-deoxyuridine (IV), has been active against colon cancer in a preliminary clinical trial but has not been found to possess any advantage over FU.[10]




R = deoxyribose

IV

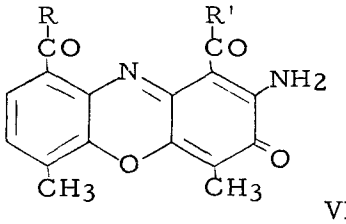
1-β-D-arabinofuranosyl cytosine (ara-C, cytosine arabinoside (V) is a synthetic nucleoside analogue with antitumor and antiviral activities; the preclinical studies on this compound have been reviewed by Smith.[11] Ara-C is active in the treatment of acute leukemia.[12] This agent produces cytotoxic and therapeutic effects by inhibiting synthesis of DNA; a nucleotide metabolite of ara-C impedes conversion of cytidylate to deoxycytidylate thus depleting cellular pools of this DNA constituent. The recent demonstration that metabolites of ara-C may be incorporated into DNA or RNA may also be pertinent to the drug's cytotoxicity.[13,14] Insensitivity or resistance to the toxic effects of ara-C has been correlated with the absence of (or loss of) deoxycytidine kinase.[14] Ara-C is rapidly deaminated to ara-U which lacks cytotoxic activity; this deamination can be inhibited by replacement of the 4-$NH_2$ with -NHOH, -$NHCH_3$, -$NHNH_2$, or -NHAc[15] or by methylation on the 5 position.[16] Such substitutions alter rates of degradation in vivo but have not produced an increase in therapeutic index. 1-β-D-arabinofuranosyl-5-fluorocytosine (ara-FC) (VI) was synthesized in the hope that it might combine the inhibitory effects of both ara-C and FUdR.[17] Ara-FC has proved to be identical in its biologic activity to ara-C in preclinical studies.[18]



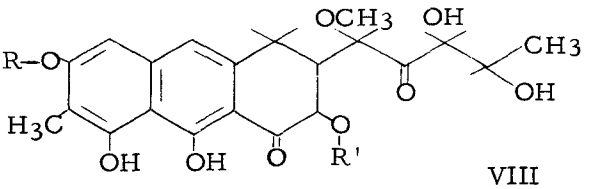

| | $R_1$ | $R_2$ |
|---|---|---|
| V | H | arabinose |
| VI | F | arabinose |

Antibiotics with Antineoplastic Activity - A number of tumor-inhibitory antibiotics form stable complexes with DNA thereby interfering with synthesis of RNA. Three of these antibiotics, actinomycin, mithramycin and daunomycin (which is probably identical to rubidomycin) possess

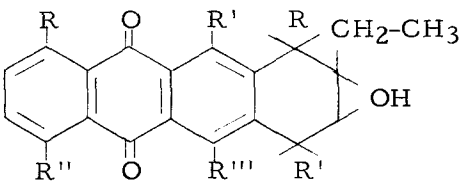
unequivocal activity against cancer in man. Kersten, Kersten and Szybalski have summarized the literature on the binding of a number of these antibiotics to DNA and reported their own observations on the physicochemical properties of the complexes so formed.[19] By chromophore groups these antibiotics can be divided into three classes: actinomycins (VII), chromomycins (VIII) including mithramycin and olivomycin, and the anthracyclines (IX) which include daunomycin, cinerubin, nogalamycin



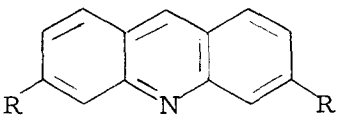

VII




VIII




IX




X

and a number of others. The binding characteristics of these antibiotics can be usefully compared with those of acridine dyes (X). The anthracyclines and acridine dyes are similar in that they intercalate between base pairs in DNA; they differ in that the anthracycline-DNA complex is stable whereas the acridines are displaced in solutions of high ionic strength. Presumably the aminosugar residues in the anthracyclines are responsible for stabilization of the antibiotic-DNA complex. Physiocochemical evidence of intercalation is not seen in complexes of DNA with actinomycins or chromomycins. Both actinomycins and anthracyclines cause stabilization of native DNA to thermal denaturation; the anthracyclines are considerably more effective in this respect on a molar basis while chromomycin antibiotics are without effect. The effect of the antibiotic-DNA complex upon thermal denaturation seems pertinent to some aspects of the drugs biological effects on cells. Separation of strands of DNA is a presumed prerequisite for their replication; an antibiotic complex which interferes with this splitting would inhibit DNA synthesis. Mithramycin has no effect upon thermal stability of DNA and no acute effect upon DNA synthesis at concentrations which inhibit synthesis of RNA.[20] The anthracyclines enhance the thermal stability of DNA to a greater extent than do the actinomycins; the former are also less selective in their inhibitory effects on synthesis of nucleic acids.[21] The relationship of binding of

these antibiotics to the base composition of DNA has been examined by means of spectral shift, change in melting profile, inhibition of RNA polymerase and the change in buoyant density in a cesium chloride gradient. The results obtained by various workers employing these several techniques are not in total agreement. It seems clear, however, that the presence of deoxyguanosine residues in DNA is required for binding of Actinomycin D. Anthracyclines can complex with poly dAT and poly dGdC; however, binding of nogalamycin may be favored by dAT pairs while that of daunomycin is not. The presence of cations ($Mg^{++}$ or $Cs^{+}$) may play a role in complex formation between the chromomycin antibiotics and DNA.

Extension of these comparisons into intact cells and into clinical pharmacology is now becoming possible at least with respect to actinomycin D, daunomycin and mithramycin. Actinomycin D is the most potent of the three drugs on a molar basis whether considered in terms of growth inhibition in cell culture, depression of RNA synthesis or the usual clinical dosage. In cell cultures, actinomycin can produce acutely almost complete inhibition of RNA synthesis; this degree of inhibition can not be achieved acutely with mithramycin[20] or daunomycin.[22] The extreme sensitivity of nucleolar function and of the synthesis of ribosomal RNA to the effects of low concentrations of actinomycin D has again been emphasized by ultrastructural[23] and ultracentrifugal[24] observations. Nucleolar morphology and the synthesis of nucleolar RNA also seem quite susceptible to the effects of daunomycin.[22,25] The existence of genetically functional DNA within mitochondria gives importance to a report that actinomycin D, mithramycin, rubidomycin and nogalamycin can each interfere with the utilization of ATP generated by oxidative phosphorylation in Sarcoma 37 ascites cells.[26] In clinical trials, both actinomycin D and mithramycin are active in some patients with testicular cancer; both drugs produce regressions in neuroblastoma.[27] On the other hand, daunomycin is active against acute leukemia while actinomycin seems ineffective.[27] In their clinical toxicology, the three drugs are similar in that they all produce bone marrow hypoplasia. They differ in that hepatic and renal toxicity, hypocalcemia and hemorrhagic phenomena, the latter related to capillary injury and elevated prothrombin time, seem peculiar to mithramycin.

With the recent availability of $^{3}H$-actinomycin D a positive correlation has been reported between uptake[28] and prolonged retention[29] of the labeled drug ty tumor cells and the sensitivity of those cells to the cytotoxic effects of the antibiotic.

Recent reports on a number of other antitumor antibiotics merit mention because of their pertinence to the study of normal cell metabolism although the drugs in question either have little clinical applicability or are yet to receive adequate trial. Cycloheximide and related glutarimide

antibiotics are receiving increasing use as inhibitors of protein synthesis in yeast and mammalian cells; Vanek and Voncracek have presented a detailed description of their biogenesis in Streptomyces noursei[30] while Siegel, Sisler and Johnson have examined the relationship between structure and fungitoxicity in Saccharomyces pastorianus.[31] Grollman has postulated an analogy between cycloheximides and emetine, and suggested a structural basis for their inhibitory effects upon protein synthesis.[32] Pactamycin also has proven to be an inhibitor of protein synthesis; differing in some respects both from puromycin and the cycloheximide antibiotics.[33,34] Kajiwara and coworkers using synchronized cultures of HeLa cells have identified a point of inhibition of cellular proliferation by phleomycin following completion of DNA synthesis and prior to prophase.[35]

Vinca Alkaloids - Because of the clinical utility of vinblastine and vincristine in the treatment of some types of human cancer, other alkaloids similarly obtained from Vinca rosea Linn. have been studied in experimental tumors and in man. Vinleurosine, a dimeric indoline alkaloid, like vinblastine and vincristine, but clearly different from them on physicochemical and chemical tests, received a therapeutic trial in 42 patients with a variety of neoplasms.[36] Only one, a patient with Hodgkin's disease, obtained clinical benefit; the majority of lymphomas and leukemias treated in this study were known to be resistant to vinblastine or vincristine. On the other hand, a clinically modified vinblastine, vinglycinate sulfate, desacetyl vinblastine 4(N,N-dimethyl-glycinate) sulfate, or VGL has produced beneficial responses in Hodgkin's disease, lymphosarcoma, bronchogenic carcinoma and chondrosarcoma.[37] Clinical improvement was obtained in several patients felt to be resistant to vinblastine and vincristine. The toxicity of VGL resembles that of vinblastine; leukopenia was dose limiting, neurotoxicity was not observed in the 31 patients studied. No better explanation for the antitumor effects of the vinca alkaloids has been adduced than that of mitotic arrest, offered some years ago; further support for this mechanism has been obtained from studies in patients with Hodgkin's disease, lymphosarcoma, and acute leukemia.[38] On the other hand mitotic arrest cannot account for the peripheral nerve injury commonly produced in patients by therapy with vincristine, nor for the inhibition of synthesis of DNA produced in regenerating rat liver by vinblastine.[39] Studies continue upon the effects of the vinca alkaloids upon protein and nucleic acid metabolism in vitro.[40] The drug concentrations employed to produce inhibitory changes in these studies are so far beyond any that are achieved in vivo that the observations are of doubtful pertinence to clinical effects.

Asparaginase - L-asparagine amidohydrolase (E.C. 3.5.1.1) inhibits growth of some murine lymphomas and leukemias; it is presently undergoing preliminary clinical trial. In leukemic patients, a drug-associated

decline in circulating leukemic cells has been observed.[41] The antitumor activity of asparaginase was first described as an unknown factor in guinea pig serum by Kidd.[42] The identity of the guinea pig serum factor was established as asparaginase by Broome.[43] In clinical trials of asparaginase an enzyme preparation from E. coli is being employed; this bacterium produces at least two proteins with asparaginase activity only one of which is active in the mouse lymphoma assay.[44] Presumably the enzyme is cytotoxic by reducing extracellular asparagine levels and thus intracellular asparagine in those cell populations which lack the enzyme to aminate aspartic acid. Asparagine dependence is not uncommon in murine leukemias.[45] When studied in vivo the earliest effect of asparaginase upon the sensitive murine lymphoma 6C3HED was inhibition of protein synthesis, as manifest in decreased incorporation of $^{14}$C-valine into protein.[46] This occurred within minutes following administration of the enzyme; inhibitory effects upon synthesis of DNA were detected by 120 minutes while rates of RNA synthesis began to fall only after 240 minutes. Extensive necrosis of tumor cells was observed by 12 hours following drug administration. The availability of an active preparation from E. coli has permitted initial clinical trial, however, the current preparation is pyrogenic and antigenic. The rate of production of the enzyme in a clinically useful preparation is still too modest to permit an adequate clinical trial.

Miscellaneous Synthetic compounds - Procarbazine, (N-isopropyl-α-(2 methylhydrazino)-p-toluamide hydrochloride), one of several derivatives of methylhydrazine which have antineoplastic activity against transplanted tumors, has proven clinically useful in the management of Hodgkin's disease. A second derivative, N-allophanoyl-α-(2 methylhydrazine)-p-toluamide, appeared to have activity comparable to that of procarbazine in a preliminary clinical trial.[47] The mechanism by which procarbazine and related compounds produce cytotoxicity is not established. Procarbazine produced a short-lived inhibition of synthesis of DNA, RNA and protein when studied in a mouse lymphoma;[48] the methyl group enters the 1-carbon pool and participates in transmethylation reactions.[49] Pertinence of these observations, as well as those previously described of molecular autoxidation to the drug's antitumor effects, awaits demonstration.

Because of the utility of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) (XI) in the study of experimental leukemia in mice as well as because of its activity against human neoplasms,[50] a series of haloalkyl nitrosoureas have been synthesized and tested against L-1210 leukemia in mice.[51] In general the most active compounds were 1-[2-(chloro or fluoro)ethyl]-1-nitrosoureas substituted in the 3 position by a 2(chloro or fluoro)ethyl or cycloaliphatic group.

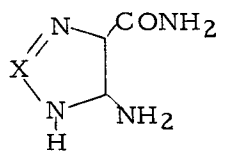
$$ClCH_2CH_2N(NO)CONHCH_2CH_2Cl$$

XI

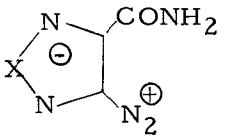
A series of acetylenic carbamates have proven to have potent antitumor effects against transplanted murine tumors and to be devoid of hypnotic properties;[52] one of these, 1-1-diphenyl-2-propynl cyclohexylcarbamate is undergoing clinical trial. In structure-activity studies the acetylenic group and aromatic 1, 1 substituents were essential for antitumor activity. Provided that the carbamoyl nitrogen had two substituents they could be varied widely. The pairing of cyclohexyl and H gave high potency.

Investigation of hydroxyurea and related derivatives has continued in spite of their limited clinical utility. That hydroxyurea is cytotoxic by reason of its inhibitory effects upon synthesis of DNA, has been widely demonstrated. Recent observation in cell culture[53] and cell-free extracts [54] have confirmed previous reports that the drug inhibits ribonucleotide reduction. A detailed morphologic analysis of cell damage in rats treated with hydroxyurea indicated that lethal effects occurred only in those cells synthesizing DNA at the time of drug administration.[55] A variety of hydroxyurea analogues have been tested in transplanted tumor systems; [56, 57] the 1-ethyl and 1-methyl derivatives of hydroxyurea possess antineoplastic activity equal to or greater than the parent compound. Oxamyl hydroxamic acid inhibited rates of growth and DNA synthesis in E. coli and Ehrlich ascites tumor respectively in a fashion quite comparable to hydroxyurea but it also produced significant methemoglobinemia which mitigates against its clinical use.[58]

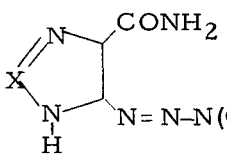
The tumor-inhibitory activity of analogues of purine and pyrimidine bases led Shealy and several coworkers to synthesize a series of analogues of the imidazoles in the biosynthetic pathway to purine ribonucleotides and to test these analogues for antineoplastic effects.[59] Beginning with 5 (or 4)-aminoimidazole-4 (or 5)-carboxamide (AIC) (XII) or the analogue 5-amino-v-triazole-4-carboxamide (XIII) they produced diazo derivatives (XIV) which react with secondary amines to produce triazenes. A number of these triazeno derivatives possess antitumor activity against transplanted cancer in animals; one compound 5-(dimethyltriazeno)-imidazole-4-carboxamide (XV) is undergoing clinical trial. Of 11 mono-substituted triazeno imidazoles tested, only 5-methyltriazeno imidazole-4-carboxamide was consistently active against the mouse leukemia L-1210. Two of nine



XII X=CH
XIII X=N



XIV



XV X=CH
XVI X=N

triazeno-v-triazole-4-carboxamide derivatives were active against L-1210; the effectiveness of the dimethyl derivative (XVI) was roughly comparable to that of the imidazole derivative (XV). In patients, XVI was found to be distributed into total body water; roughly 50% of an oral dose was absorbed; and 40% of an intravenous dose was excreted within 6 hours.[60] The drug is a potent marrow suppressive in animals and man.[61]

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Section IV - Metabolic Diseases and Endocrine Function
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## Chapter 17. Antidiabetic Agents

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This review is essentially a continuation of the same chapter in the 1965 Annual Reports. Its primary purpose is to extend and up-date the material presented there. Both reviews emphasize physiology and biochemistry because the significant advances in diabetes research are being made on these fronts, while progress toward new antidiabetic drugs has been slow. This emphasis also stems from a conviction that new approaches to antidiabetic therapy are most likely to come from application of new knowledge about metabolism and its regulation.

There have been two general reviews of diabetes.[1,2] The latter, by Goodner, is particularly recommended for its comprehensive treatment of the biochemistry and physiology underlying metabolic regulation and the way these processes are deranged in diabetes.

### Insulin

"Too much is known about insulin", complains a Lancet editorial writer,[3] and surveying the mass of information that is yet to be fitted into a coherent, unifying theory of insulin secretion and action, one is inclined to agree. Reviews on the pancreatic β-cell,[4] the chemistry and biochemistry,[5] biosynthesis,[6] secretion and action of insulin,[7] published during 1966, all leave the impression that far more is known than is understood.

Biosynthesis and Storage. Available evidence leaves little doubt that insulin, like other polypeptides, is assembled in linear fashion from the N-terminal end on a ribosomal RNA template. The chief question has been whether the two chains are produced independently or whether a proinsulin, like chymotrypsinogen, is first formed and then cleaved into the two chains after the disulfide linkages have been formed. Current evidence favors the view that the A and B chain are produced independently. Combination of the two chains apparently does not involve a specific enzyme and the proper alignment of the sulfhydryl groups seems to be directed solely by the configuration of the polypeptide chains.[6] Glutathione insulin transhydrogenase, which can catalyze the oxidative coupling of the two chains, apparently has no capacity to direct the establishment of a particular configuration.[8] The morphology of insulin storage, as revealed by electron microscopic studies, has been reviewed recently.[4]

Secretion. Despite the vast and rapidly growing body of fact about insulin secretion, there is still little insight at the molecular level into mechanisms by which various substances trigger insulin release. It certainly seems that the time is ripe for unification and correlation of this knowledge and the formulation of hypotheses that can serve as a basis for further experiment. The tools exist. Many substances are now known to cause the release of insulin from the pancreatic β-cell, several of which appear to do so in different ways. There are also a number of inhibitors of insulin secretion and techniques exist for studying the action of these substances on pancreatic preparations ranging from isolated cells to the perfused organ.[9,10,11]

Recent evidence suggests that insulin is not a feedback inhibitor of its own secretion.[12]

Glucose is one of the major natural stimulants of insulin secretion. It acts directly on the β-cell and apparently must be metabolized to exert its effect. Other metabolizable sugars, such as fructose and mannose, also stimulate insulin release but non-metabolizable sugars do not.[11,13] Inhibitors of glucose metabolism such as mannoheptulose[9] and 2-deoxyglucose[13,14] prevent glucose from stimulating insulin release. The effect of glucose is so rapid that detectable elevations of plasma insulin occur within one minute of starting a glucose infusion. In the isolated perfused rat pancreas, a glucose pulse produces a pulse of released insulin within 30 seconds.[15] Calcium is essential to the process and cannot be replaced by magnesium. Potassium is also apparently essential and a sufficiently high potassium concentration can cause insulin release from isolated pancreas preparations.[15] Pretreatment with growth hormone or with glucocorticoids leads to an increased response of insulin secretion to glucose, but neither hormone has an appreciable direct effect on *in vitro* preparations.[7,9]

Glucagon is also a potent, direct stimulator of insulin secretion[10,13,17] and the suggestion has been made that its action may be mediated through 3',5'-AMP, which has been found to stimulate insulin secretion directly.[18] Considering the mechanism of the glycogenolytic effect of glucagon in the liver, this is a reasonable suggestion, which is consistent with results of experiments in man (see below).

The insulin releasing activity of the sulfonylureas has been studied extensively and differs in important respects from that of glucose. Mannoheptulose and diazoxide do not inhibit the insulin releasing effect of the sulfonylureas although they both block glucose induced insulin secretion.[9,19]

The insulin releasing effect of amino acids has been thoroughly reviewed.[20] Many, but not all, amino acids stimulate insulin secretion to some extent. Arginine, because it is the most potent, has been studied extensively. The action of leucine differs sufficiently from that of the other amino acids that it appears to function by a different mechanism. Arginine is a potent stimulator of insulin secretion in the normal pancreas

and its effect is not blocked by diazoxide or by epinephrine. Pretreatment with growth hormone increases the sensitivity of the pancreas to arginine, as it does to glucose. The effects of leucine are somewhat different. Although leucine causes significant insulin release from the normal pancreas, effects comparable to those obtained with arginine are achieved only on hyper-functioning pancreatic tissue - either in patients with functioning islet cell tumors or in subjects stimulated by three days of chlorpropamide pretreatment. The insulin releasing effects of leucine in chlorpropamide pretreated subjects can be blocked by diazoxide.

Adrenergic mechanisms are of great interest in insulin release, not only because of their probable importance to the physiological control of insulin secretion, but also because the generally high level of understanding of adrenergic mechanisms should lead, by analogy, to an improved understanding of the mechanisms that govern insulin release in the pancreas. Predominantly α-adrenergic stimulants such as epinephrine and norepinephrine inhibit insulin release and their effects can be partially blocked by α-adrenergic blocking agents such as dihydroergotamine and phentolamine.[21] The β-adrenergic-stimulant, isoproterenol, on the other hand, causes insulin secretion from the pancreas and this effect can be almost completely blocked by the β-adrenergic blocking agent, propranolol.[22] Considering these effects, and that 3',5'-AMP stimulates insulin release directly, it is natural to suggest that the cyclic nucleotide may be one of the mediators of insulin secretion. Enhancement of insulin secretion by theophylline, a phosphodiesterase inhibitor, supports this idea.[19] Moreover, propranolol, which is known to inhibit isoproterenol but not glucagon activation of adenyl cyclase, blocks isoproterenol hyperglycemia and insulin release, but does not inhibit either the hyperglycemia or insulin release caused by glucagon in human subjects.[22]

The hyperglycemic effects of diazoxide are well documented and are known to result from an inhibition of glucose uptake in peripheral tissue as well as inhibition of insulin release from the pancreas.[19] Diazoxide has no effect on insulin release by the sulfonylureas or arginine,[20] but blocks glucose and leucine[20] stimulation of insulin secretion. Diazoxide inhibition of insulin secretion can be at least partially reversed by α-adrenergic blocking agents.[19] Whatever the primary action of diazoxide, it is apparently sufficient to overcome its phosphodiesterase inhibitory action,[23] which would be expected to potentiate insulin secretion, as theophylline does. Considering the known effects of diazoxide on intracellular electrolyte balance,[23] it would be interesting to examine this aspect of its action in connection with effects on insulin release. That potassium is essential for insulin secretion and can even stimulate the process, has been mentioned. Convincing evidence has been offered that carbohydrate intolerance that may well derive from insufficient insulin secretion is frequently associated with clinical states of potassium deficiency, especially primary aldosteronism.[24]

Insulin Action. A comprehensive listing of the actions of insulin is given by Williams and Ensinck in their review.[7] The specific effects of insulin on plasma carbohydrate and lipid turnover have also been re-

viewed.[25] Steiner has reviewed the effects of insulin on metabolism in the liver and has included a thoughtful analysis, insofar as one is now possible, of the relative effects of substrate availability, enzyme synthesis and allosteric effects.[26] Weber and his colleagues have reviewed the concept that insulin functions over the relatively long term to control the metabolic activity of the liver by acting as an inducer of glycolytic enzymes and a repressor of gluconeogenic enzymes.[27] Other reviews on the control of liver metabolism emphasize the shorter term feedback control exerted by metabolic substrates.[28,29,30,31] Some new data on the effects of acute and chronic insulin deprivation on hepatic metabolism are also valuable.[32,33]

Levine currently views the action of insulin as occurring primarily at the cell membrane, but certainly not as a primary effect solely on glucose transport, from which all other effects derive.[34] He makes the important point that intracellular effects do not necessarily mean intracellular presence. Indeed few, if any, of the postulated actions of insulin have been produced in cell free systems and there remains no convincing evidence that insulin penetrates beyond the cell membrane. Although there is now good evidence that insulin can stimulate protein synthesis independently of amino acid transport facilitation, it has not been possible to demonstrate a direct effect of insulin on a complete cell free protein synthesizing system in vitro.[35,36] The effect of insulin treatment or insulin deprivation on the animal (or isolated perfused liver) from which the preparation is made, are readily seen, however. Potassium balance in muscle has proved to be remarkably sensitive to insulin. In muscle preparations, ion fluxes can be seen after treatment with insulin at levels that produce no detectable effect on glucose metabolism.[37] Insulin rapidly reduces 3',5'-AMP levels in adipose tissue, probably by inhibiting adenyl cyclase, and this effect has been suggested as the basis of the direct, antilipolytic action of insulin.[38] A provocative recent paper presents evidence suggesting that during insulin activated glucose transport across the rat fat cell membrane, the sugars form either imines or glycosylamines with the ε-amino group of lysine residue.[39]

Two new theories of insulin action have been offered and commented on.[3] Bessman has suggested that insulin acts by forming a mechanical link between hexokinase and the mitochondrion, thus promoting efficiency by anchoring an ATP requiring process near its source of supply.[40] Dormandy has suggested, from experiments using erythrocytes and hemoglobin, that insulin has an immediate physical action on the cell-extracellular interface, altering the redox potential gradient across the plasma membrane.[41]

Plasma Factors that Influence Insulin Action. Berson and Yalow argue persuasively that there is no evidence that insulin of pancreatic origin ever becomes bound to a plasma protein, from which it can subsequently be released in recognizable form.[42] Newer evidence, based on an examination of the insulin-like activity of plasma, from which all immunoreactive insulin had been precipitated with anti-insulin serum, confirms this conclusion.[43] Nevertheless, there is ample evidence that there exist in plasma, substances that have insulin-like activity or that can modify the

action of insulin in experimental systems. There now seems to be general acceptance of the view that bound insulin (Antoniades), atypical insulin (Samaan and Fraser), and nonsupressable insulin (Froesch) are the same. Indeed, there is as yet no convincing evidence to rule out the possibility that such insulin-like activity is also identical to the Vallance-Owen insulin antagonist.[44] The two classes of substances have generally been regarded as different because they are prepared in different ways and there has been a tendency to explain certain similarities in their action by postulating cross-contamination.[45] A line of experimentation that adopted the view that the two substances were the same and then attempted to upset this hypothesis, might yield some very interesting results. Both materials inhibit insulin uptake by isolated muscle and adipose tissue, inhibit the action of insulin on muscle (diaphragm) and show insulin-like activity on adipose tissue.[45] In vivo activities of both preparations have been demonstrated by injection of the partially purified factors into rats.[46,47,48] Some of these, employing intraperitoneal injections and examination for effects on the diaphragm, appear to be special cases of in vitro experiments, however, since the diaphragm was surely directly exposed to appreciable concentrations of the injected material.[48] The existence of both an albumin insulin antagonist and bound insulin has been demonstrated in rat plasma.[49] The level of plasma nonsupressable insulin has been found not to decrease during supression of insulin secretion by mannoheptulose.[50] Additional evidence has been obtained that the sulfonylureas do not release insulin from bound insulin preparations.[51]

Several groups are studying the Vallance-Owen insulin antagonist from various points of view. Purification leading to 150 fold enrichment has been carried out.[52] Reproducibility of preparations of the antagonist from plasma has often been poor[53,54] and the use of alternate albumin isolation methods has led to fractions with insulin-like activity but no inhibitory activity.[54] Further confirmation has been obtained that the albumin fraction from diabetic plasma contains consistently more inhibitory activity than that from nondiabetic subjects.[53] Preparations obtained to date are still far from homogeneous and have been shown to contain α-globulins.[53] From their studies on the mechanism of action of the Vallence-Owen insulin antagonist, Davidson and Goodner conclude that its primary effect is to block the sugar transport effect of insulin and that albumin bound free fatty acids play no part in its action.[55] There is still no conclusive evidence to indicate whether the Vallance-Owen insulin antagonist, as had been postulated earlier, is the albumin complex of the insulin B-chain, but prepared mixture of reduced B chain and albumin did increase hyperglycemia in rats made diabetic by a high protein, high fat diet.[56] The separate A and B chains both inhibited glucose uptake in diaphragm, but neither blocked the stimulatory effect of added insulin. The A chain, but not the B chain, increased the incorporation of glucose carbon into $CO_2$ in adipose tissue. This action could be blocked by anti-insulin serum.[57]

Evidence has been offered for the existence of a hormone, produced in the liver and under pituitary control, that favors lipogenesis from glucose by inhibiting the action of insulin on muscle.[58] A substance that

stimulates the uptake of nonutilizable sugars by the rat diaphragm, reminiscent of the Goldstein work factor, has been isolated from the gas perfused cat heart.[59]

## Other Hormones

The pituitary growth hormone is known to exert profound effects on metabolism and pancreatic function. Although the hormone has little effect on acute administration or on tissues *in vitro*, continued administration leads to substantially elevated plasma insulin levels and increased insulin secretion in response to a glucose load.[60] A theory that the anabolic effects of growth hormone require the additional effect of insulin and that, with relative deficiency of insulin, its diabetogenic action (elevated free fatty acid levels and decreased glucose penetration) predominates has been proposed.[61] The role of growth hormone as a pathogenic agent in diabetes has been questioned recently, partly as a result of studies on patients who underwent pituitary stalk section to relieve retinopathy. This is taken as further confirmation that whether growth hormone is diabetogenic probably depends on whether there is an adequate supply of insulin.[62]

Glucagon and epinephrine acutely stimulate gluconeogenesis in perfused rat liver, probably through 3,'5'-AMP. The hormone sensitive step is apparently before the formation of three carbon intermediates, since gluconeogenesis from fructose is unaffected.[63] The effect of catecholamines and other hormones on lipid mobilization has been well reviewed by Steinberg.[64]

## Intermediary Metabolism

One of the most important products of research on diabetes has been increased understanding of the physiology and biochemistry of metabolic regulation. It is obviously impossible, in a review of this kind, to cite more than a few of the advances that have been made, even in a single year. The selection here is based on a judgment of their significance to diabetes, heavily weighted by the personal interest of the reviewer. Several have already been mentioned in connection with hormone activities.

Randle has reviewed his concept of a glucose-fatty acid cycle, with some new experimental material.[65] There has been a recent review of gluconeogenesis, with good current references.[66] The control of phosphofructokinase, one of the important rate limiting enzymes of glycolysis, is still not fully understood. The activity of the enzyme in mammalian muscle is influenced by substrate concentration and by 3',5'-AMP, which can activate the ATP inhibited enzyme, apparently by an allosteric effect. Activation by epinephrine has not been demonstrated, however, and other adenine nucleotides may well be involved.[67] Glucokinase, previously found in rat liver, has now been identified in extracts of liver from well nourished humans and dogs, but is absent during periods of poor nutrition.[68] Fatty acids have been found to inhibit several liver glycolytic enzymes and

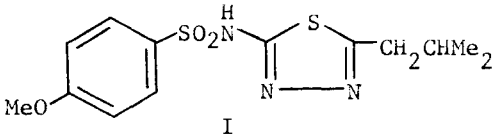
especially pyruvate kinase. A scheme whereby this effect could act to change the metabolic activity of the liver from glycolysis to gluconeogenesis has been proposed.[69]

The regulation of fatty acid synthesis in adipose tissue has been reviewed by Ball, with emphasis on a citrate-malate cycle that provides both extramitochondrial acetyl-CoA and part of the NADPH required for fatty acid synthesis.[70] Further quantitative work on the pathways of glucose and acetate carbon in adipose tissue has also been reported.[71]

## Sulfonylureas and their Congeners

The introduction to therapy of tolazamide[72] brings to four the number of sulfonylureas or related compounds commercially available in the United States. A comprehensive investigation of the metabolism and excretion of acetohexamide in human subjects has largely confirmed the conclusions reached in earlier studies and the report of this work includes leading references to studies on the metabolism of other sulfonylureas.[73] 1-Butyl-3-(p-hydroxymethyl)benzenesulfonylurea has been identified as a significant metabolite of tolbutamide in man and the predominant metabolite in the rat.[87] In a pilot clinical evaluation, 1-cyclohexyl-3(4-trifluoromethyl-benzenesulfonyl)urea gave satisfactory diabetic control with dosage once a day.[74] An investigation of the hypoglycemic activity and toxicity of a hypoglycemic sulfathiadiazole (I) has been published.[75]

A series of exceptionally potent sulfonylureas has been reported. Threshold hypoglycemic doses of the most active compound (II) in the rat and dog were only 0.005-0.020 that of tolbutamide. Hypoglycemic activity at 0.1 mg/kg in humans was reported.[76] A number of piperazinesulfamylurea congeners of an earlier series of sulfamylureas have been synthesized.[88]

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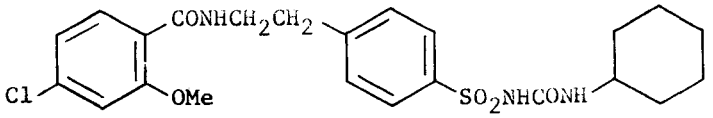


II

Further studies have confirmed that prolonged sulfonylurea treatment does not lead to exhaustion of the pancreas, but slightly increases its insulin secreting capacity.[77]

## Biguanides

When writing about phenformin and the other biguanides, authors almost without exception state that the mechanism of the hypoglycemic

action of these drugs is unknown. The weight of evidence now available leads one to question seriously whether this disclaimer is still warranted.

It now seems reasonable to accept, as was proposed some years ago, that the primary action of phenformin is to inhibit energy transfer at the cytochrome b site of the energy transport chain[78,79] and that this results in decreased ATP concentrations and increased inorganic phosphate and AMP concentrations. The known effects of phenformin on metabolism can rationally be traced back to this primary effect on electron transport. The most important of these effects on metabolism are increased glycolysis in adipose tissue and muscle and reduced gluconeogenesis in the liver. Both can be accounted for by the resulting reduction in the ATP/AMP ratio, which regulates the activity of the key enzymes of glycolysis, gluconeogenesis and oxidative metabolism in ways that are now reasonably well understood. Whether hypoglycemia results in a given circumstance will depend on the balance of the effects on glycolysis and gluconeogenesis--that is, how rapidly the lactate formed by glycolysis in fat and muscle is reconverted to glucose in the liver. The operation of this balance of effects has been elegantly demonstrated by Altschuld and Kruger.[80] In both *in vivo* and *in vitro* experiments, they showed that the guinea pig, which is quite sensitive to the hypoglycemic effects of DBI, responds to the drug with decreased liver ATP levels and marked reduction in gluconeogenesis from lactate and glycerol. The rat, on the other hand, which is quite insensitive to the hypoglycemic effects of DBI showed no significant reduction of either ATP levels or gluconeogenesis. In recent studies, which he regards as preliminary because of the small number of patients involved, Kreisberg found that phenformin inhibited gluconeogenesis (observable as a reduction in the absolute quantity of glucose recycled and a reduction in glucose replacement rate).[81]

Several objections to this mechanism for the hypoglycemic action of the biguanides were discussed in last year's review. Some additional comment on these is appropriate here. The evidence that the hypoglycemic action of the biguanides depends ultimately on an inhibition of oxidative processes rests heavily on the results of *in vitro* experiments. It has frequently been objected that the concentration of the biguanides necessary to produce the observed effect is substantially greater than is obtained *in vivo*. In one recent study, however, using isolated tissues from the guinea pig, the rat and the pigeon, the drug concentration required to inhibit oxidative processes was essentially the same as that required to stimulate glucose uptake and the concentrations were in the range that drug metabolism studies suggest are attained *in vivo*.[82] Moreover, the relative sensitivity of the tissues was in the same order as the relative sensitivity of the three species to the hypoglycemic action of the biguanides. In another investigation, a number of the effects of phenformin on metabolism in rat adipose tissue,-- inhibition of glucose oxidation and lipogenesis, both basal and insulin stimulated-- were traced to an inhibition of pyruvate oxidation, presumably resulting from interference with electron transport.[83]

The suggestion that the biguanides potentiate the action of insulin by increasing the uptake of insulin by muscle[84] seems questionable. If this were the case, one would expect the effects of the biguanides on metabolism to parallel those of insulin, but in fact, they are usually the opposite. Insulin promotes glycogen deposition, lipogenesis, and protein synthesis, all of which the biguanides oppose.[83,85]

Convincing clinical reports that phenformin treatment results in weight loss continue to appear,[86,87] but no biochemically rational explanation for this effect has yet been advanced. Inhibition of lipogenesis has frequently been mentioned as the cause of the observed weight loss, and is often attributed to decreased plasma insulin levels. Certainly this is a well documented metabolic action of the biguanides, but it is clearly insufficient to explain weight loss. Whether dietary carbohydrate is more or less readily converted to fat can have no effect on body weight unless there is a net loss of carbon from the system. When weight loss is due to an imbalance between caloric intake and energy expenditure, the net loss of carbon is in the form of $CO_2$. Phenformin has been reported to cause an increase in net conversion of glucose carbon to $CO_2$,[86] but an attempt to confirm the observation has thus far failed.[81] Both investigations, however, are in relatively early stages and resolution of the question must await the results of further work. There could be a net loss of carbon in biguanide treated patients through urinary excretion of lactate, but there is little evidence to suggest that this occurs to a significant extent. Weight loss due to biguanide treatment has been attributed by some investigators to the anorectic effect of the drug, and considering the differences that small decrements of caloric intake can make, it is not possible to dismiss this suggestion from data presently available, especially without an alternate rationalization.

## Other Hypoglycemic Compounds

A series of 4-(1-naphthyl)butylamines, for the most part derivatives of naphthylacetic acid, have shown hypoglycemic activity comparable, in the most active members, to chlorpropamide.[89] A superficial structure-activity analysis has been attempted, but no details of their action have been published.[90]

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# Chapter 18. Atherosclerosis

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Introduction - The importance of atherosclerosis as a cause of death and disability, especially in Europe and North America has increased tremendously within the past few decades. This is partly because, with the advancement of medical science, more people live longer. Nevertheless, the development of atherosclerosis severe enough to jeopardize normal function, or even life itself, cannot be considered a normal part of the aging process. Many factors have been implicated in its genesis: diet, heredity, stress, obesity, hypertension, metabolic disorders such as diabetes and hypothyroidism, climate and smoking. Probably no one factor alone produces the degenerative changes seen in advanced atherosclerotic disease. There is circumstantial evidence that manipulation of one or several of these basic factors will benefit at least some percentage of the population and therefore these must be pursued. Preventive treatment directed solely against basic causes may be impractical and perhaps a more logical approach is to try to prevent or inhibit formation of atheromatous plaques which eventually become calcified, resulting in the characteristic lesion of arteriosclerosis.

The Framingham study continues to yield information correlating serum lipid levels with coronary heart disease. Risk of coronary disease is related to lipoproteins and their lipids.[1] This risk could be predicted at all ages for men, but only before the age of 50 for women. In diabetics excess of heart disease occurs related to elevation of lipoprotein levels, especially triglyceride-rich particles. Triglyceride and cholesterol concentrations and levels of their transporting vehicles independently exert an influence on heart disease. The same study showed that risk of angina pectoris and sudden death was proportional to the degree of overweight.[2] Adiposity, however, proved unrelated to the rate of development of myocardial infarction. The contribution of obesity to risk of angina and sudden death was independent of blood pressure and cholesterol levels. The Framingham study of 5,127 subjects can find no difference in mean cholesterol-phospholipid ratio between those who developed coronary disease and those who did not.[3] Schilling and co-workers,[4] in a study of 2,100 men and women of ages 18 to 65, found that the triglyceride curve (plotted against age) was parallel to serum cholesterol in the female while in the male it deviated. In precoronary and postcoronary patients triglyceride levels were higher than in normal subjects. They conclude that variation in levels of serum triglyceride is more closely related to coronary arterial disease than variations of serum cholesterol levels.

Further evidence has been presented that a strong association exists between smoking and atherosclerosis. Increased mortality is seen in smokers[5,6] although the correlation between smoking and serum lipid patterns is not clear. A study by the Health Insurance Plan of Greater New York[7] has shown that physical activity plays a role. The least active smoker has an incidence of rapidly fatal myocardial infarction which is nine times that found among the most active smokers. A rapid rise in free fatty acid is seen[5] and an enhanced utilization of fatty acids[8] occurs in smokers apparently due to nicotine ingestion. The relationship of this fatty acid rise to myocardial infarction is uncertain.

Diet - That serum lipid levels can be manipulated to some degree by dietary methods alone has been known for some time. The problem is the design of a diet which is acceptable to the patient. The design of such a diet has recently been discussed.[9] It has a moderate fat content (30% of calories) containing 10% saturates and 13% polyunsaturates. The effectiveness of adherence to a "prudent diet" is seen in the Anti-coronary Club study[10] which reports that the prevalence of obesity, hypertension and hypercholesteremia were significantly reduced during the first four years of this program. The authors also present data showing a decrease in new coronary disease events in the study group, although as yet these numbers are relatively small.

Engelberg[11] has shown that the introduction of highly unsaturated fats to the diet lowers triglyceride levels and increases the rate of lipolysis in normal adults. There was no change in lipolysis rate in patients in whom triglyceride levels did not decrease. This provides further evidence that lipoprotein lipase is the major physiological pathway for the removal of endogenously synthesized triglycerides from the blood stream and provides an explanation for the triglyceride-lowering action of unsaturated fats. The effect of dietary carbohydrate on serum lipid levels is under extensive study[12,13,14,15,16,17] but published information does not permit positive correlations as yet.

Correlations between coffee drinking and elevated serum lipid concentrations were found in men with coronary heart disease but not in healthy controls.[18] Caffeine has been shown to elevate free fatty acids in the human and in the dog.[19] This elevation is suppressed by the administration of sucrose. The authors feel that caffeine elevates FFA by a mechanism different from adrenaline or nicotine and may be a direct effect on fat pads and via an influence on the adrenal cortex.

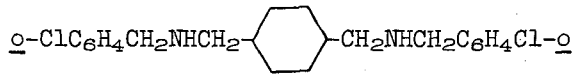
Drugs Affecting Serum Lipids - Because cholesterol is the principal component of the atheromatous plaque, measures to reduce the level of cholesterol in the blood by drug therapy may reasonably be expected to prevent, or at least retard, the development of atherosclerosis. The most important drugs available today have been reviewed recently.[20] The National Lipid Lowering Study, whose intent is to evaluate the effects of cholesterol lowering drugs on the prognosis of coronary disease, is just getting underway. The study anticipates a total of 8,000 patients

to be studied for ten years and end points to be measured are the incidence of secondary myocardial infarctions, strokes or death. The drugs entered into the program at the moment are D-thyroxin, nicotinic acid, Clofibrate (CPIB) and Premarin.

The fate of agents which inhibit cholesterol biosynthesis and accumulate sterol intermediates is still debated. No direct link between desmosterol build-up and the profound side effects of triparanol (lenticular cataracts, alopecia and ichthyosis) have been demonstrated. Recent reports have indicated that triparanol (MER-29) increased the incidence of aortic lesions in gerbils,[21] improved atherosclerotic symptoms in pigeons,[22] and in man,[23] for periods up to sixteen weeks, lowered blood pressure, levels of serum cholesterol and produced improved cardiac function. Other agents which accumulate desmosterol continue to appear. A large series of A- and B-ring-modified azacholesterols[24] and cholesterol analogs with oxa- and oxa-aza side chains[25] have been reported.

The cholesterol lowering agent AY-9944 (1) has been studied extensively in the rat,[26] pig,[26c] dog[26c] and cockerel.[27] It inhibits the conversion of 7-dehydrocholesterol to cholesterol and cholesterol levels in the serum, liver, adrenals, kidney and aorta are decreased while the precursor levels are increased.[26b] The compound has been used as a tool to study the course of cholesterol biosynthesis.[28] Study of a series of structural analogs has allowed a definition of the structural requirements for this type of activity.[29]

A large series of biphenylyl-substituted compounds was tested *in vitro* for inhibition of the synthesis of cholesterol.[30] 5-(4-Biphenylyl)-3-methylvaleric acid (W 2531) (2) and 2-(dimethylamino)ethyl 5-(4-biphenylyl)-3-methylvalerate hydrochloride (W 2795) were the most potent inhibitors. W 2531 inhibits the incorporation of acetate into cholesterol and lowers serum cholesterol in the rat[31] but not in the dog.[32]



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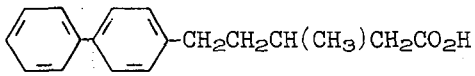

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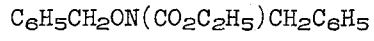


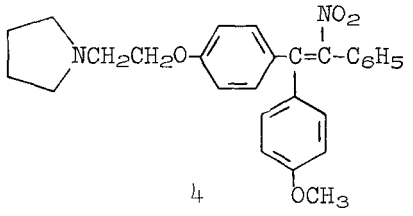
3

Studies on the action of Benzyl N-Benzyl carbethoxyhydroxamate (W 398) (3) showed an accumulation of cholesterol in the liver with no effect on serum cholesterol levels. It is suggested that the drug inhibits the transfer of cholesterol from the liver to the blood.[33]

Phenformin continues to show interesting effects on serum lipids in addition to its antidiabetic action. It produces lowered serum cholesterol and triglyceride levels in man.[34] No effect on lipolysis has been seen in rats.[35] These effects are not detectable with other antidiabetic agents such as Tolbutamide and Tolazamide.[36]

Atromid S (CPIB, ethyl chlorophenoxyisobutyrate) has now been released for sale in the U.S.A. Reports of its effectiveness in lowering serum triglycerides and serum cholesterol continue to appear.[37] It has shown effectiveness in the diabetic[38] with concomitant disappearance of retinal exudates.[38a] In combination with Thyroxine the two drugs complement one another.[39] Fibrinogen levels have been lowered dramatically when CPIB was given to patients with coronary thrombosis.[40] This effect on fibrinogen could not clearly be related to its cholesterol lowering property. The drug appears to lower free fatty acid levels in man[41] but an inhibitory effect on lipolysis in rat epididymal adipose tissue could not be detected.[42] Concern over the hepatomegaly produced by CPIB has prompted extensive study of the effects of the drug on the liver. This increased liver weight seems to disappear on chronic administration in the monkey and dog.[43] Microbodies appear in the liver simultaneously with the appearance of hypolipidemia[44] suggesting a relationship between microbodies and lipid metabolism.

Other agents which have shown activity in experimental animals include phenyramidol[45] which appears to inhibit hepatic microsomal enzymes, o-benzoylthiamine disulfide,[46] a series of pyrimidine-5-acetic acid derivatives,[47] the antifertility agent 4[47a] and chondroitin sulfate A,[48] which is said to afford protection against coronary atherosclerosis. Three butyrophenones, WY-6123 (5), WY-3457 (6) and Trifluperidol (7) were studied in humans.[49] WY-3457 had been reported earlier to inhibit cholesterol synthesis and to produce ichthyosis. WY-6123 had little effect on cholesterol levels but Trifluperidol had a marked effect in

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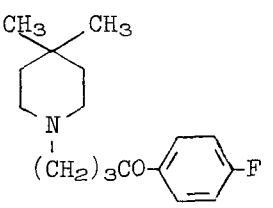

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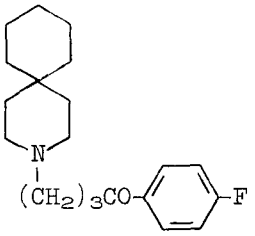

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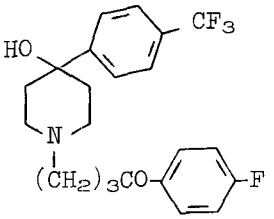

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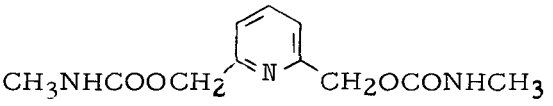

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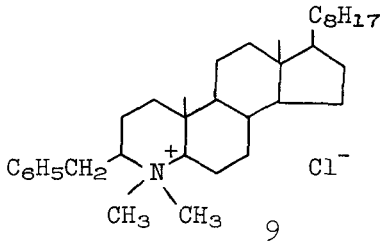
lowering cholesterol levels and after 5 months there has been a complete absence of skin and hair changes. Roniacol (β-pyridylcarbinol) when given to patients for 6 months produced a marked reduction in fatty acid esters and cholesterol.[50] Nicotinic acid produced similar results but the authors claim Roniacol gave fewer side-effects. Pyridinolcarbamate (8), an antibradikinin agent, is reported to prevent atheromatous changes in the rabbit.[51] In man[52] the agent is reported to have a definite antianginal effect along with a host of other reactions, including antiinflammatory and analgesic responses.

A very promising method of lowering serum cholesterol appears in the bile acid sequestering agents. The material which has received most clinical work, cholestyramine, an anion exchange resin, sequesters bile acids from the gut, stimulating cholesterol degradation and consequently also cholesterol synthesis. The rat[53] and pig[54] are able to synthesize cholesterol rapidly enough to compensate for this bile acid drain while the chicken and human cannot, and therefore show a decreased level of serum cholesterol and other lipids.[55] This effect can also be produced simply by T-tube drainage of the bile.[56] The drug has some effect on removal of fatty acids,[56a] thyroxine,[57] porphyrins[58] and fecal nitrogen[59] but at effective doses there appears to be no clinical problem. The development of gallstones has been reported in guinea pigs[60] with the use of this drug but at the same time neomycin, which also sequesters bile acids, is reported[61] to prevent cholestanol induced gallstones in the rabbit. Cholestyramine has found clinical use in the relief of pruritis[62] of biliary cirrhosis and psoriasis. Other agents which have been shown to remove bile acids effectively are neomycin and N-methylated neomycin,[63] thyroid hormones,[64] DEAE Sephadex,[65] and the azacholestane 9.[66]



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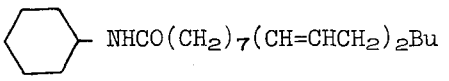

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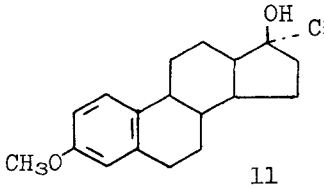
Several agents which inhibit absorption of cholesterol have also been studied as cholesterol lowering agents. These include N-cyclohexyl-linoleamide (Linolexamide) (10)[67] diosgenin,[68] pectin[69] and a variety of mucilaginous polysaccharides.[70]

The influence of lipolysis inhibitors continues to be studied vigorously but their influence on the atherosclerotic process is uncertain. Nicotinic acid and various derivatives is the subject of many communications.[71] It has been shown to prevent the FFA rise caused by smoking in humans.[72] Perhaps the most interesting lipolysis inhibitor at the present time is prostaglandin $E_1$[73] which is extremely active at a very low dose level. The effect of the β-blocking agent Propranolol has been studied in acute myocardial infarction and found to be of no value.[74] It appears to have some benefit in angina pectoris[75] but its side effects are severe.

Hormones - A more detailed account of the effect of estrogen on lipids and its protective effect on coronary atherosclerosis in the cockerel has been published.[76] This same protective effect on the coronaries is also reported in the cholesterol-fed pigeon.[77] A suggestion has been made that the female aorta under the influence of estrogen has a greater "healing-power" of the vascular connective tissue than the male aorta.[78] Evidence has been presented that patients with coronary artery disease have a higher ratio of estrone to estradiol than normals suggesting that estradiol may indeed be a protecting factor in the disease.[79]

The search for estrogens having a greater effect on lipids than on the uterus continues.[80] 16α-chloro-estrone-3-methyl ether has shown no effect in humans[81] while the steroid 11 appears to have a rather large split between its hypocholesteremic and uterotropic responses in the rat.[82] The various esters of nicotinic acid with estradiol have been studied extensively but appear to be uterotropic.[83]



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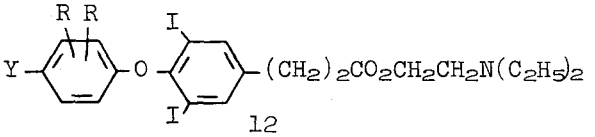

12

A series of thyroxine analogs having structure 12 have been synthesized and evaluated for hypocholesteremic activity.[84] No promising candidates are apparent. α-Methyl $DT_3$ and $DT_4$ have been prepared but show only a weak hypocholesteremic response in mice.[85] Negative reports on the effectiveness of thyroxine are beginning to appear. Cholesterol fed cockerels are not protected against coronary atherosclerosis by D- and L-Thyroxine,[86] and in fact the thyroid hormone may enhance the development of cholesterol atherosclerosis in the rabbit despite a lowering of serum cholesterol.[87] A very preliminary report also finds no change in mortality or morbidity in humans treated with D-thyroxine.[88]

The role of antiinflammatory drugs in the treatment of atherosclerosis is still being investigated. Cortisone acetate has been shown to reduce the development of plaques in cholesterol-fed rabbits by about 75%.[89] Phenylbutazone was also effective but aspirin was not. Cortisone did not cause regression of already formed plaques indicating that the action of the drug is in the early stages of plaque formation through its antiinflammatory properties rather than through any lipemic effects.

Thrombosis and Fibrinolysis - Platelet adhesiveness is mediated by ADP. Release of ADP from the platelet causes aggregation.[90] This response is inhibited by ATP.[91] The mechanism proposed for the clumping reaction[91] is that ADP inhibits the splitting of ATP to ADP, a reaction which otherwise occurs through platelet membrane ATPase which usually keeps the platelet in an unsticky state. Inhibition of this ATPase by ADP leads to the exposure of adhesive sites and permits platelet aggregation.

Certain physiologic and dietary materials affect the platelet reactions. Catechol amines enhance platelet aggregation[92] and promote thrombus formation.[93] Fatty acids appear to enhance platelet aggregation, with saturated fatty acids being more effective than unsaturated fatty acids.[94] Thrombosis is produced in the dog by single rapid infusion of long-chain saturated fatty acids[95] but chronic slow infusions are nonthrombogenic.[96] Apparently the dog has sufficient thrombolytic power to handle the latter situation.

Literature on the effects of dextran on platelet adhesiveness is contradictory. The method of measurement is important. It appears to reduce platelet adhesiveness to glass[97] but has no effect on ADP-induced aggregation.[98] Its effect in humans is equally confused.[99] Although it may play a role in surgery[100] and certain occlusive vascular diseases,[101] it appears to have no benefit in acute myocardial infarction.[102]

Atromid S reduces platelet adhesiveness[103] and increases bleeding time[104] in man. Estriol, in man, has no effect on platelet adhesion.[105] A large series of guanidino compounds has been studied and found to be active inhibitors of ADP-induced platelet aggregation.[106] Some of the most active are δ-guanidinovaleric acid, p,p'-diguanidinodiphenylsulfone and p,p'-diguanidinodiphenylmethane. Other agents which have shown some activity are N-Ethylmaleimide[107] and a series of membrane-active drugs,[108] such as chlorpromazine, imipramine, nortriptyline, etc. Prostaglandin $E_1$

has been shown to reduce clotting time very markedly in rats.[109]

A study in coronary patients has shown that fibrinogen levels are increased over normal controls[110] and that fibrinolytic activity is also decreased in these patients.[111] The rate of degradation of fibrinogen is inhibited in rats fed an atherogenic diet.[112] It would seem reasonable then that some control over the clinical outcome of atherosclerosis could be had by the use of fibrinolytic agents. The enzymes streptokinase and urokinase continue to be evaluated[113] with some benefits reported in the therapy of acute Thromboembolism.[114] Phenformin[115] is still the only drug of interest as a fibrinolytic agent other than the enzymes and CPIB mentioned earlier.

Psycho-Social Factors - One's personality and environment greatly influence his prognosis of coronary thrombosis.[116] Personality type A individuals are highly prone while the fully developed type B are essentially immune to the disease[117] provided their serum lipids are not abnormal. Seemingly healthy individuals exhibiting type A behavior exhibit a serum lipid pattern similar to that found in persons suffering from coronary artery disease. They exhibit abnormal preprandial and postprandial values of serum triglycerides while blood sugars are normal.[118] This triglyceride response can be controlled by the administration of corticotropin,[119] which acts apparently by augmenting the adrenal discharge of hormones. The mechanisms involved in the lipid responses of behavior type A remain uncertain.

Summary - Knowledge in the field of atherosclerosis is expanding at a very rapid rate. There seems to be sound evidence that vigorous dietary measures along with the use of drugs can bring serum lipid levels to a "normal" value and that, with these measures, a considerable majority of coronary heart disease and atherosclerosis cases may be prevented. The Anti-coronary Club study lends support to this statement and hopefully the National Lipid Lowering Study, just getting underway, will provide conclusive evidence on this point.

The release of Atromid S to the U.S. market provides a powerful tool to the physician in his fight against coronary artery disease. A very promising method of treatment is emerging in the use of bile acid sequestering agents. This therapy provides safety along with a method of lowering cholesterol levels which is highly physiologic in nature.

Study of the mechanism of thrombosis and its inhibition by drugs affecting platelet aggregation is proceeding at a rapid rate and shows great promise for future application. The application of Prostaglandin $E_1$, a material occurring naturally in humans, in this area is an intriguing possibility. The study of atherosclerosis and its prevention remains one of the most vigorous and perhaps one of the most important areas in medical research.

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# Chapter 19. Reproduction

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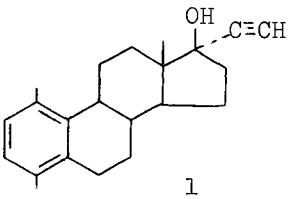
Introduction - At first sight, the intricate process of mammalian reproduction would appear vulnerable to interuption by outside agencies at numerous points. In the female, for example, one could, at least in principle, prevent ovulation, sperm capacitation, transport of that sperm to the oviduct, penetration of the egg by sperm, division of the fertilized egg, transport of the egg to the uterus, or even finally nidation. The developmental process of the male germ cell from the spermatogonium to a mature sperm cell would also appear to offer numerous points of attack. Despite this, the only practical means of chemical contraception as of this writing consists of estrogen progestin type of oral contraceptives. Though much effort has been expended in the search for some means of contraception which does not involve the pituitary, such an agent has not yet been found.

Steroids - The steroidal oral contraceptives now available in the U. S. have recently been reviewed[1] as has the chemical development of these drugs.[2] The belated recognition of the importance of the estrogen component of these formulations has led to the development of the so called sequential agents; studies seem to indicate that better control of intramenstrual bleeding is achieved with the "sequentials,"[3,4,5] than with the combination drugs. The finding that certain progestins had long lived action when administered parenterally has led to the development of injectable long acting contraceptives; one of these (medroxyprogesterone acetate) is administered as the pure progestin,[6] the other (dihydroxyprogesterone acetophenide) is given with an injectable estrogen (estradiol enanthate).[7] Interestingly, some clinical success has been realized in achieving contraception with an orally administered pure estrogen (diethyl stilbestrol)[8] or a pure progestin (chlormadinone) in low dosage.[9]

Apparent exceptions to the rule that a saturated A ring is necessary for progestational activity have been reported recently. Thus, compound 1 was shown to exhibit weak activity in the Clauberg assay[10] while 2 showed good activity in inducing arborization of rabbit uterine epithelium;[11,12] the observation that 2 is uterotropic as well,

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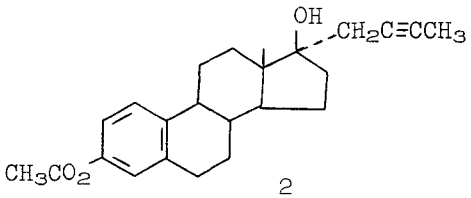


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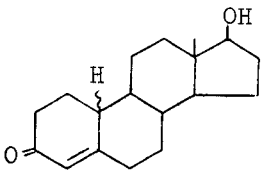
suggests that a single compound estrogen-progestin contraceptive is in the realm of possibility.

The search for steroids with non-classical biological spectra included the preparation of the modified steroids 3 to 10. The biological activities are summarized in Table I.

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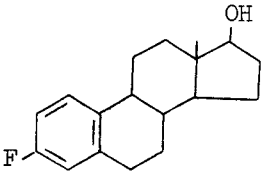


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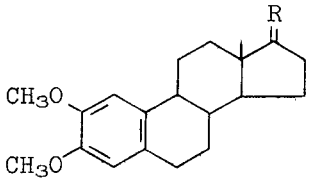
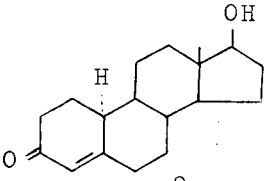



5; $\Delta^{8,14}$, R = O
6; R = O
7; R = OH, H

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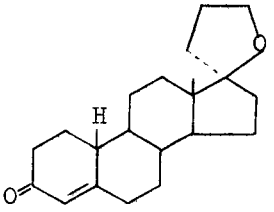


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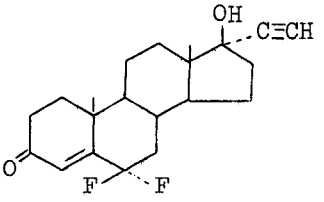
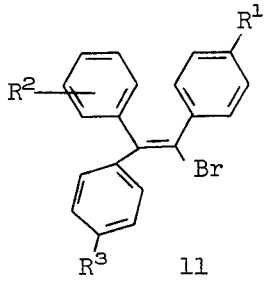



10

Table I
Biological Activities of Selected Steroids

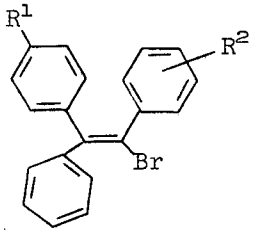
| Compound | References | Biological Activity |
|---|---|---|
| 3 | 13 | 5% of Progesterone; Clauberg |
| 4 | 14 | very weak estrogen |
| 5 | 15 | uterotropic; .01% esterone |
| 6 | 15 | uterotropic; .03% esterone |
| 7 | 15 | uterotropic; 0.6% esterone |
| 8 | 16 | gonatropin inhibitor; low uterotropic activity |
| 9 | 17 | antiestrogen; antiprogestin, |
| 10 | 18 | progestin |

Compounds Related to Triphenylethylene - Derivatives of this class of synthetic estrogens continue to be an area of interest for the preparation of potential antifertility agents. Thus, compounds 11,[19] 12,[20] and 13[21] are all reported to possess uterotropic activity, the potency depending on the substitution pattern. No biological data are available for 14.[22] The incorporation of a basic ether into the nitro triaryl ethylene 15[23] led to a potent oral antifertility agent; this compound (CN-55,945-27) was shown to be an estrogen antagonist[24] and to be effective probably because of this antagonism.[25] Replacement of the nitro group in 15 by an ethyl similarly leads to an estrogen antagonist. In this case, however, the cis and trans isomers (16, 17)[26] showed markedly different biological properties; the cis compound (ICl 47, 699; 17) behaved as a conventional estrogen while the trans compound (ICl 46, 474; 16) was a weak estrogen and antagonized the effect of concomittantly administered estrogen.[27] In puzzling contrast to this, the isomers of 15 show identical antifertility activity,[23] and the corresponding isomers of 12 show the same antigonadotropic potencies.[20]



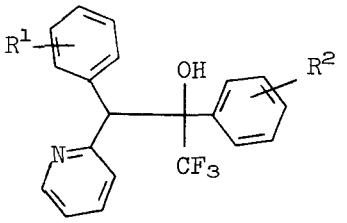

11

$R^1$ = H,$CF_3$
$R^2$ = $CF_3$
$R^3$ = $CH_3$,$OCH_3$



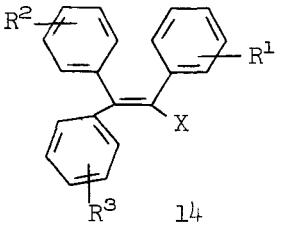

12

$R^1$ = H,OH,$OCH_2C_6H_5$
$R^2$ = F,Cl,$CF_3$



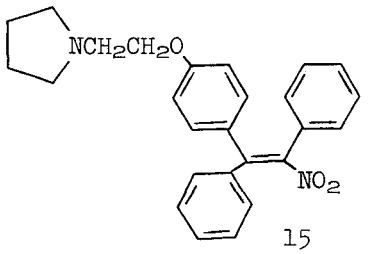
13

$R^1$ = H,$OCH_3$
$R^2$ = H,Cl,$OCH_3$

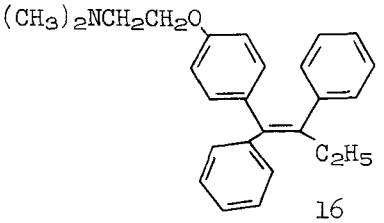


14

$R^1$,$R^2$ and/or $R^3$ = $OCH_3$
X = Br,Cl



15



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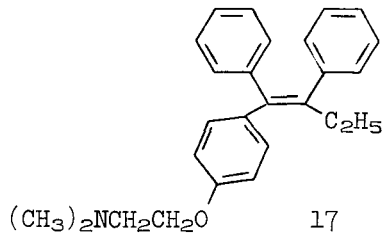
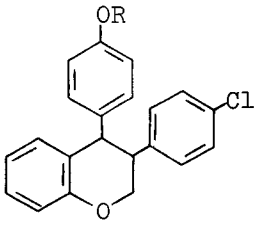
trans



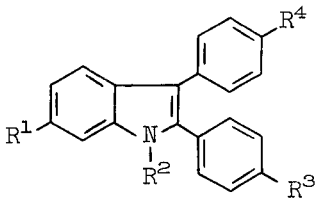
17

cis

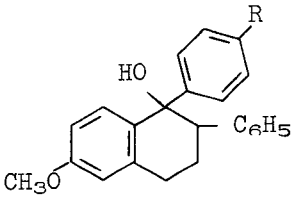
A series of chromenes and chromans (18, 19 and 20), analogous to previously recorded tetrahydronaphthalenes,[28] has been reported;[29] compound 19 [cis, R=$OCH_2CH_2N(CH_2H_5)_2$] was the only one to show activity as an antifertility agent at 1 mg./kg. Replacement of a methylene by nitrogen similarly leads to attenuation of potency; thus, only one [23, $R^1$=$R^2$=$R^4$=H; $R^3$=$OCH_2CH_2N(C_2H_5)_2$] of a series (21-24) of indoles showed antifertility activity.[30] The tetrahydronaphthols 25 and the dihydronaphthalenes represented by 26 were all shown to be potent estrogens;[31] in contrast to this, the glyceryl ether 27 showed many of the estrogen antagonistic properties characteristic of the previously reported basic ether.[32] Attempts to simplify these molecules by replacement of the phenyl at the 1 position by vinyl and ethynyl (28) led to a series of inactive compounds.[33]



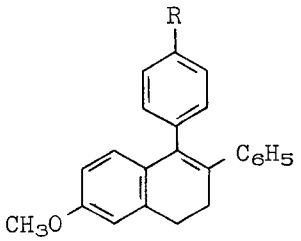

18; $\Delta^{1,2}$; R = H,$CH_3$,$CH_2CH_2N(C_2H_5)_2$
19; cis diphenyl; R = H,$CH_3$, $CH_2CH_2N(C_2H_5)_2$
20; trans diphenyl; R = H,$CH_3$, $CH_2CH_2N(C_2H_5)_2$



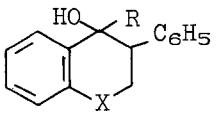

21; $R^1$ = $OCH_2CH_2N(C_2H_5)_2$; $R^2$,$R^3$,$R^4$ = H,$OCH_3$
22; $R^2$ = $OCH_2CH_2N(C_2H_5)_2$; $R^1$,$R^3$,$R^4$ = H,$OCH_3$
23, $R^3$ = $OCH_2CH_2N(C_2H_5)_2$; $R^1$,$R^2$,$R^4$ = H,$OCH_3$
24; $R^4$ = $OCH_2CH_2N(C_2H_5)_2$; $R^1$,$R^2$,$R^3$ = H,$OCH_3$



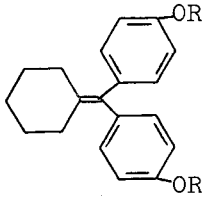

25; R = H,$CH_3$,F




26; R = H,$CH_3$,F,OH
27; R = $OCH_2CHOHCH_2OH$




28; R = $-CH=CH_2$,$-C\equiv CH$
X = $CH_2$,O

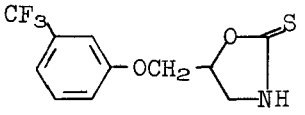



29; R = H,$OCOCH_3$

The cyclohexylidene 29, has been reported to be a weak estrogen, effective as an antifertility agent, which will under certain conditions exhibit antiestrogenic activity.[34]

Miscellaneous Structural Types - The oxazolidine thione 30 (U-11,634) was reported to be an effective antifertility agent in the rat.[35] It was shown to be devoid of hormonal properties and to suppress the deciduomata development in a traumatized uterus.[36] The dibenzodiazocine 31[37] caused complete inhibition of fertility in rats at 2.5 mg./kg. in the

rat; the demonstration[38] that this compound behaves as an estrogen on several endpoints seems to account for this activity. A uterotropic factor from spoiled grain[39] has been isolated; structural elucidation



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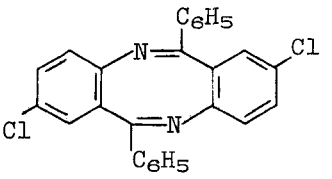

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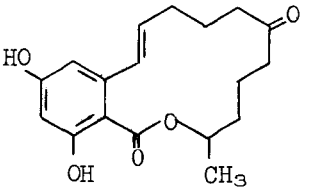

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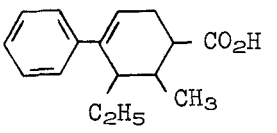

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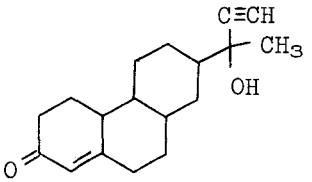

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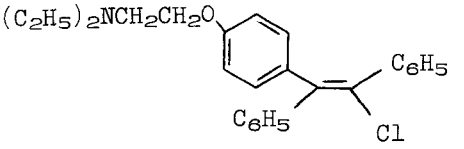

35

showed this compound (zearalenone) to be the macrocyclic lactone 32.[40] This class of compounds has been reported capable of controlling animal fertility.[41] A potential approach to a postcoital "pill" comes from the finding that the nonsteroidal estrogen 33 effectively prevented pregnancy in the rhesus monkey when administered at 10 mg. per day for 6 days following mating.[42] The perhydro phenanthrene 34 (Ro 5-2537) shows interesting steroid antagonistic properties in that it will inhibit the uterotropic effect of stilbestrol but only minimally that of estradiol; the compound also inhibits the stimulatory effects of androgens in the male.[43] The possible significance of such an agent in reproductive processes is not known.

Induction of Ovulation - Fallout from the recent emphasis on work on antifertility has been a tool for the treatment of infertility due to ovulatory failure. The use of clomiphene citrate (35) for this clinical condition has met with a good measure of success;[44] a double blind study demonstrated that the response was indeed a drug effect.[45] Though the exact mechanism of action of this agent is not clear, it has been shown to increase the estrogen output in women without increasing urinary gona-

dotropins.[46] An alternate approach to induce ovulation involves the use of human gonadotropic preparations.[47,48] Refractory cases have been successfully treated with a combination of clomiphene and gonadotropins.[49]

Biological Considerations - Progress in this area as in many others depends on developing a clearer understanding of finer details of the hormonal mechanisms involved in both the normal processes and in drug action. The availability of radioimmune assays for human serum LH[50,51] and for urinary FSH[52] should help provide information on the action of drugs at the pituitary level. In an elegant piece of work, the uterine estrogen receptor was recently isolated in highly concentrated form.[53]

The role of the hypothalmus in the regulation of the reproductive cycle is coming under increasingly intensive investigation; the action of hormones on this entity has recently been reviewed.[54] Considerable progress is being achieved towards the isolation of the hypothalamic releasing factors for the pituitary hormones.[55]

Summary - The means for chemical contraception are now at hand, with various estrogen progestin regimes. Considerable clinical and biological effort is devoted to finding new and more convenient means of employing these hormonal agents. The success achieved with postcoitally administered estrogens may lead to a postcoital pill. Despite a large amount of both chemical and biological work on compounds which may be loosely termed estrogen antagonists, no notice of clinical success of such an agent has as yet appeared. An ideal agent for achieving contraception would be one which completely bypassed the endocrine system; this breakthrough is still to come. The intensive work on the endocrinology of reproduction is bringing closer the day when the process will be fully understood.

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Chapter 20. Steroid Hormones and their Antagonists

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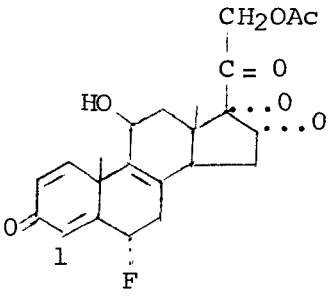
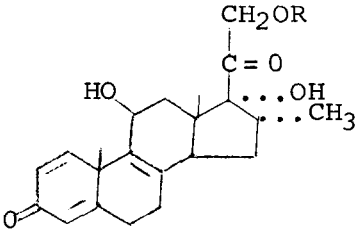
A bibliography of steroid conjugates[1] as well as a treatise on the metabolism of steroid hormones[2] have been published. The abstracts of papers presented at the Second International Congress of Hormonal Steroids are available[3].

## Corticoids

A possible explanation for the anti-inflammatory action of cortisol based on the prevention of release of plasma kinins has been published[4]. A number of 8-dehydro analogs of corticosteroids have been synthesized[5] and their thymolytic and salt retaining properties measured. The compound found to have the highest thymolytic activity was 1 (11.4 x hydrocortisone). Again the fallacy of projecting biological activity from one series of steroids to another was demonstrated by the large decrease in activity exhibited by the 8-dehydro steroid 2 as compared with 16α-methylprednisolone (3).

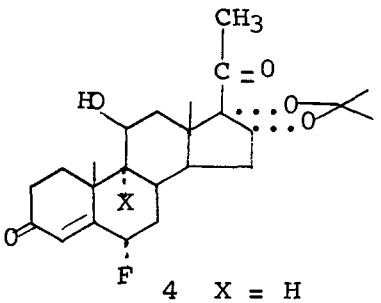


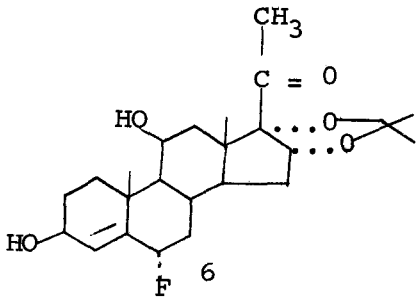

2 R = Ac

3 R = H, 8,9-dihydro

The 21-desoxy-16α,17α-isopropylidenedioxypregnenes 4 and 5 show potent anti-inflammatory properties and the allylic alcohol 6 has water solubility and a partition coefficient (water-ether) which might predict a favorable percutaneous absorption[6].



4 X = H
5 X = F



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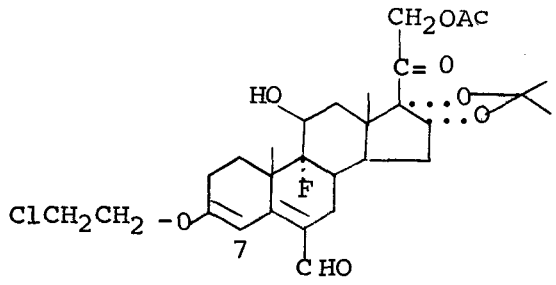
A number of 3-(2'-chloroethoxy)-6-formylpregn-3,5-dienes have been prepared[7] of which 7 shows a marked dissociation of local and systemic anti-inflammatory activity. Both 1,4-dimethylestratrienes 8 and 9 are active in the carrageenin-induced foot edema rat assay at the subcutaneous dose level of 25 mg./rat[8].



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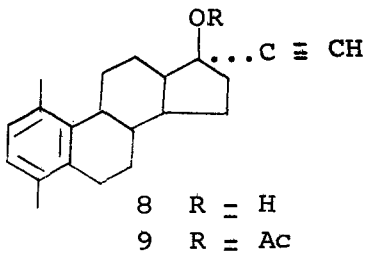


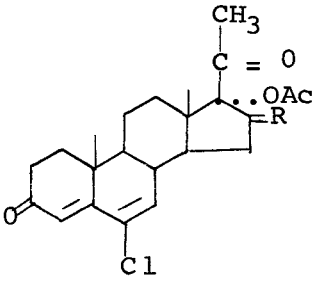
8 R = H
9 R = Ac

It has been proposed[9] that there is a direct relationship between the inhibition of growth of mouse fibroblasts in vitro and the anti-inflammatory activity of 11β-hydroxylated steroids. 11-Ketosteroids do not show this inhibition and this may be due to the lack of enzyme systems necessary to convert 11-keto to 11β-hydroxy steroids[10].

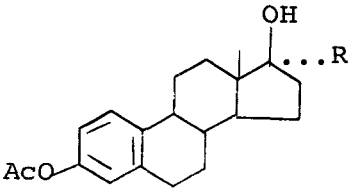
Studies on the mechanisms regulating secretion of aldosterone and glucocorticoids[11] and on the mode of action of aldosterone[12] have been published.

## Progestational Compounds

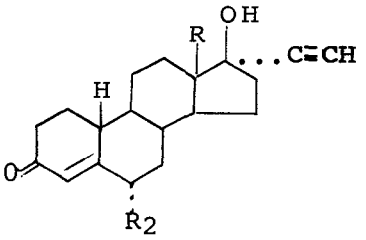
The 16-methylene derivative (10) of chlormadinone (11) is twice as active as 11 in the modified Mc Phail assay on oral administration[13]. The deciduogenic activity of substituted estradiols has been used as another parameter for assessing intrinsic progestational activity[14,15,16]. Both 17-(2-butenyl)-estradiol 3-acetate (12) and the 17-(2-methylallyl) derivative (13) were active in this assay. The totally synthetic 18-homolog 14 of norethisterone (15) has proven to be a potent progestational compound in animals[17,18] and in man[19,20,21]. The 6α-methyl derivative 16 has 85 times the activity of 15 in the Clauberg assay[22]. (±)-18-Methyl-19-norprogesterone (17) is a potent progestogen[23].



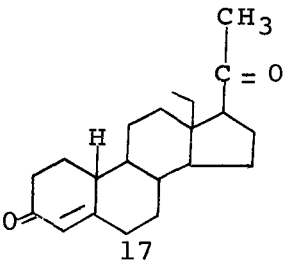
10 R = $CH_2$
11 R = $H_2$



12 R = $CH_2CH{=}CHCH_3$
13 R = $CH_2C(CH_3){=}CH_2$



14 $R_1$ = $C_2H_5$, $R_2$ = H
15 $R_1$ = $CH_3$, $R_2$ = H
16 $R_1$ = $C_2H_5$, $R_2$ = $CH_3$



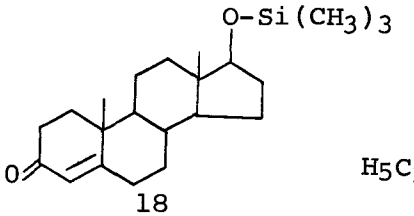
17

Androgens

The effect of 1-alkyl substitution in decreasing the androgenicity of a number of 5α-androstanes has been noted and a proposal regarding the structural requirements for biological activity of androgens has been made[24].

17β-Trimethylsiloxytestosterone (18) has been shown[25] to be more active on subcutaneous administration than testosterone. The prolonged duration of action of this compound has also been noted[26].

A new class of orally active alkyl steroid-17β-yl mixed ketals of aliphatic and cycloaliphatic ketones have been prepared[27]. A member of this series, 17β-(1-methoxy-1-cyclohexyloxy)-3-propionyloxy-5α-androst-1-ene (19) shows greater androgenic activity than methyl testosterone.

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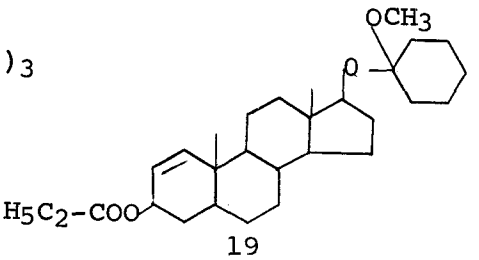
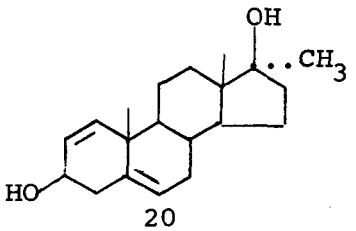



19

An evaluation has been made of the nitrogen retention: androgenic and myotrophic:androgenic dissociation ratios of anabolic steroids[28]. A number of new anabolic agents have been synthesized which have a good ratio of myotrophic to androgenic activity. These include 17α-methyl-androsta-1, 5-diene-3β,17β-diol(20)[29], 5α-estr-2-en-17β-ol (21)[30] and its 17α-methyl derivative 22, the two 2α,3α-epithio-5α-androstanes 23 and 24[31], the 1-keto-5α-androstane 25[32] and 17β-(1-methoxy-1-cyclohexyloxy)-androst-5(10)-en-3-one (26)[27].

17α-Methyl-4,9,11-estratriene-17β-ol-3-one(27), a potent anabolic steroid, has been reported to produce liver toxicity with low dosage in humans[33].

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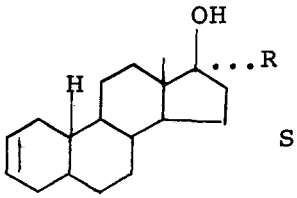
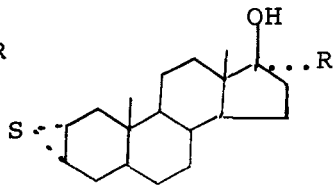
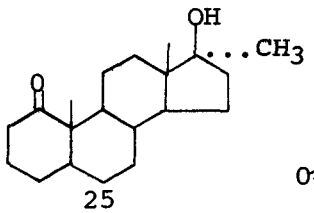



21 R = H
22 R = $CH_3$




23 R = H
24 R = $CH_3$

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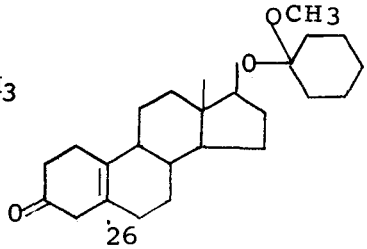


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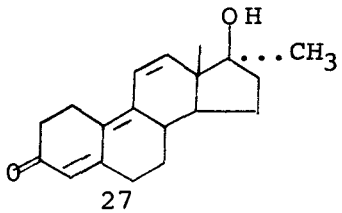
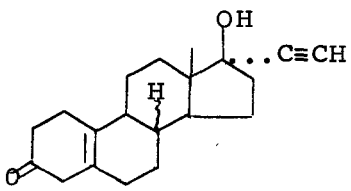



27

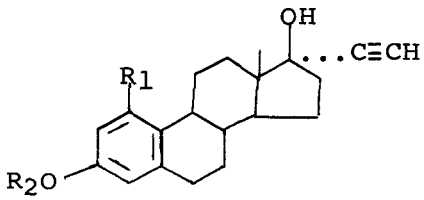
Estrogens

The relative uterotrophic activities of 3- and 17-desoxy derivatives of estrone and estradiol and their alkyl derivatives have been published[34]. A comparison of the hormonal activities of some new 17α-haloethynyl steroids is also available[35].

The totally synthetic (±)-8α-isomer 28 of 17β-hydroxy-17α-ethynylestr-5(10)-en-3-one (29) is about one-half as active as 29 in a pituitary blockage test[36]. 1-Acetoxy-17α-ethynylestradiol 3-acetate (30) is ten times as active orally as 17α-ethynylestradiol (31)[37].

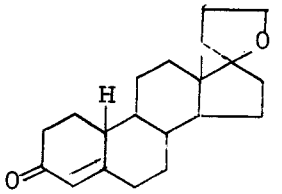



28 8α
29 8β




30 $R_1$=OAc $R_2$=Ac
31 $R_1$=$R_2$=H

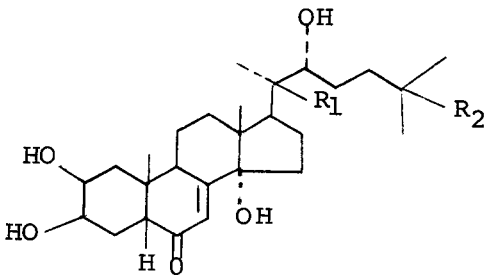
Estradiol dipropionate has been shown to hasten brain maturation in new born rats[38]. It has been found that there is no necessary correlation between estrogen antagonism and progestational activity of steroids[39]. The spiran 32 exhibits very pronounced antiestrogenic activity[40].




32

Insect Hormones

The synthesis of ecdysone (33) the insect moulting hormone has been announced by two groups[41,42]. Furthermore isolations of 20-hydroxy-ecdysone (34) from silk worm[43] (ecdysterone)[44], crayfish[45] and oak-silk moth (crustecdysone)[46] and tobacco hornworm[47] have been reported. Four compounds have been isolated[48] from the leaves of Podocarpus nakaii Hay all of which have insect moulting activity. The structure proposed for ponasterone A is 35. Crustecdysone has been isolated from Podocarpus elatus, an Australian timber tree[49]. The ready isolation of these compounds from plants makes it possible to supply large amounts of active substances for biological testing.

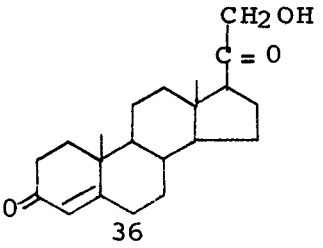



33 $R_1$ = H $R_2$ = OH

34 $R_1$ = $R_2$ = OH ($C_{20}$ and $C_{22}$ configurations undefined)[22]

35 $R_1$ = OH ($C_{20}$ and $C_{22}$ configurations undefined)
$R_2$ = H

The secretion of large amounts of cortexone (36) by the water beetle (Dytiscus maaginalis) as a defensive substance has been reported[50].

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# Chapter 21. Non-steroidal Antiinflammatory Agents

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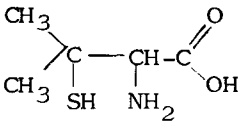
Divison of Merck & Co., Inc., Rahway, New Jersey

Introduction - Interest in the search for new antiinflammatory drugs has continued with growing emphasis. During the past year more than one hundred groups of new compounds have been disclosed in the patent literature as potential antiinflammatory agents. The prospect of finding a better drug, possibly with a novel mode of action, is further enhanced by the intensive clinical and pharmacological efforts to unravel the etiology and pathogenesis of rheumatic diseases.[1] An increasing appreciation of various immunological factors involved in many connective tissue diseases[2] has opened a new dimension to medicinal chemists. A wealth of background materials are to be found in two comprehensive monographs[3,4] and several review articles.[5,6,7] Current clinical and biological reports are conveniently abstracted in an NIH publication[8], freely available to qualified investigators in this field.

Etiology and Pathogenesis of Rheumatic Diseases - The detection of various infectious agents, e.g. PPLO[9] and diphtheroids[10], in the synovial joints of some rheumatic patients continues to suggest that chronic symptoms may be the result of a primary infection with immunologically induced secondary manifestations. Following an acute inflammatory response the pathogenesis is initiated by the presence of an antigenic material, such as denatured γ-globulin or other autoantigens[11], the formation of which may involve sulfhydryl exchange reactions. An insoluble immuno complex is then formed with an antibody like rheumatoid factor, possibly with the participation of complement. The antirheumatic activity of sulfhydryl compounds, e.g. penicillamine (I), may be attributable to its ability to dissociate

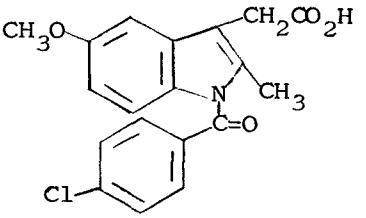


I

or inactivate the immuno macro globulins at this stage.[12] The ensuing chemotaxis and phagocytosis processes have also been investigated in detail. The former is inhibited by hydrocortisone and chloroquine in vitro[13], and the occurrence of extensive phagocytosis in synovial joints is evidenced by the observation of "inclusion bodies" inside polymorphonuclear leukocytes.[11,14] An outcome of phagocytosis is the release of various lysosomal proteins and enzymes[15], which are capable of producing local inflammation, tissue erosion,

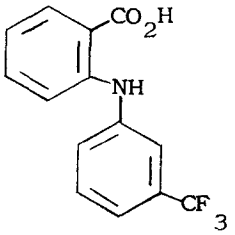
protein denaturation and the generation of autoantigens, and thus completing a self-perpetuating cycle of pathogenesis.

Test Methods - As a model manifesting both an acute inflammatory response and disseminated secondary lesions, the adjuvant arthritis assay in rats has become widely accepted in many laboratories. The effect of various known agents on such parameters as the decrease in foot volume, the restoration of body-weight loss, the reduction of plasma "inflammation units"[16], and the change in erythrocyte sedimentation rate has been described.[17,18,19] The relative potency of known drugs was estimated by Glenn[20] as indomethacin (II), 81, hydro-

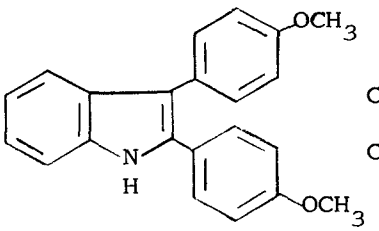


II

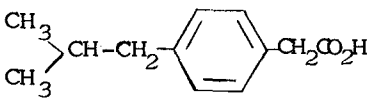
cortisone acetate 6, flufenamic acid (III) 1.6, indoxole (IV) 1.1,



III



IV



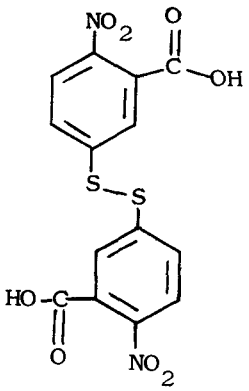
V

phenylbutazone 1 and ibufenac (V) 0.5. With certain immune suppressive agents, Newbould[21] observed that the gradual development of secondary lesions (polyarthritis) is inhibited by a three-dose treatment started just before the challenge with Freund's adjuvant.

The procedure of inducing inflammation in canine joints with microcrystalline sodium urate has been modified and extended to man.[22,23] When needed, this assay provides another animal species for preclinical evaluation of new drugs.

With the availability of new non-steroidal agents as reference compounds, the development of new in vitro assays as potential primary screens has been much facilitated. According to Mizushima[24], the thermal denaturation of protein can be inhibited by a number of antiinflammatory compounds with an order of activity roughly in agreement with their in vivo potency. As a simple model to study the stabilization of lysosomal membrane, Brown[25] has described a new

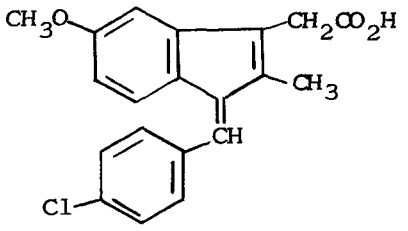
protocol based on heat-induced hemolysis of canine erythrocytes. The interaction of antiinflammatory acids with the lysyl $\epsilon$-amino group in serum albumin or histidine decarboxylase, in competition with pyridoxal phosphate, was suggested by Whitehouse[26] as a mode of action for these agents and adoptable as a preliminary assay. It is interesting to note that the lysyl $\epsilon$-amino group has also been implicated in the cross-linking of fibrins[27] and collagen (after oxidation to aldehyde).[28] Alterations in the reactivity and serum level of free sulfhydryl groups have previously been related to connective tissue diseases. An in vitro assay measuring the acceleration of disulfide interchange between serum sulfhydryl groups and 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB, Ellman's reagent VI) by non-steroidal agents was recently described.[29] The increased rate may be indicative of free sulfhydryl groups uncovered by antiinflammatory agents through an induced conformational change of the protein molecule. However, Hichens[30] observed that antiinflammatory compounds can block the binding site of DTNB, which has a configuration not unlike several antiinflammatory aryl acids. Thus, the acceleration may be caused by an increase of unbound DTNB concentration instead. Studies on the inhibition of various tissue proteases by known drugs have been extended.[31] Several antiinflammatory acids have also shown fibrinolytic activity in vitro at relatively high concentrations.[32] This is coincidental with a recent clinical note suggesting the application of fibrinolytic agents in rheumatoid arthritis.[33]



VI

So far, no single in vitro assay has been generally accepted as the most promising preliminary screening. Many antirheumatic drugs have shown activity in a variety of the in vitro assays mentioned above. Obviously the spectrum of a compound and not the activity in any single assay should be emphasized. At the present time, the optimal biochemical profile of an ideal agent remains shrouded in the unknown.

Indomethacin and Analogs - Among several isosteric modifications of indomethacin studied at Merck[34], an indene isoster, "cis"-1-p-chlorobenzylidenyl-5-methoxy-2-methyl-3-indenylacetic acid (VII), was found to be half as active as indomethacin. The corresponding "trans" isomer was only one-tenth as active. The structure-activity relationship of $N_1$-aroyl and $N_1$-aralkyl indole acetic acids is generally parallel to that of indene isosters, indicative of a similar mode of action. Based on X-ray diffraction data, the nonplanar configuration of the cis-isomer was suggested as the preferred conformation of

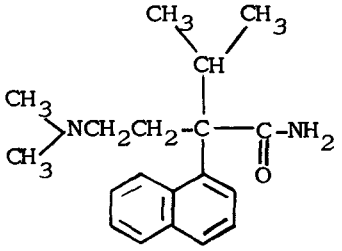


"cis" VII

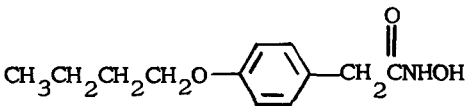
indomethacin (II) in vivo.

The possible modes of action of indomethacin remain to be a subject of active interest. As a reference compound it has been found to be active in most of the in vitro and in vivo assays mentioned above. While none of these can be considered as the primary mode of action, certainly several are contributory. An earlier report[35] of kallikrein inhibition was not confirmed.[36] The suggestion[37] that the antiinflammatory activity of indomethacin may be due to the displacement of tissue-bound corticosteroid has also been disputed.[38] The distribution and metabolism of indomethacin in various species have been reported in detail[39], and a number of long-term clinical studies have been published.[40-43]

Aryl acetic Acids - Of several aryl acetic acids described in the last report, no significant developments have been noted regarding ibufenac and namoxyrate. Detailed pharmacology and toxicity data on naphthypramide (VIII)[44] and p-n-butoxyphenylacethydroxamic acid (CP 1044 J3, IX)[45,46] have been published. The antiinflammatory potency



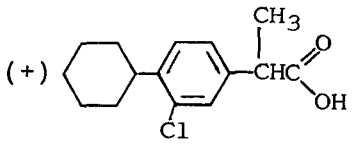
VIII



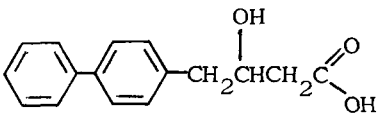
IX

of the latter is comparable to phenylbutazone and is at least partly attributable to its effects on the adrenals. The corresponding amide has analgesic activity only, and the free acid is completely inactive.

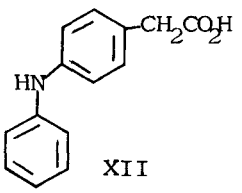
Available animal data indicated that several substituted phenyl aliphatic acids possess a high degree of antiinflammatory activity, greater than or comparable to that of indomethacin. In the carrageenin edema assay the $ED_{50}$ of (+) 3-chloro-4-cyclohexyl-α-methylphenylacetic acid (X) is ca. 0.3 mg/kg.[47], being the most potent compound reported so far. 4-Biphenylyl-3-hydroxybutyric acid (XI) has an $ED_{50}$ of 14 mg/kg. in the edema assay and a potency of six times phenylbutazone in the U.V. erythema assay.[48] Another analog, p-anilinophenylacetic acid (XII)[49] also showed a high degree of



X



XI

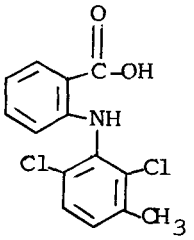


XII

activity in both edema and adjuvant arthritis assays. The ortho anilino analogs (see fenamates below) are less active. Substituted 3-phenothiazinyl acetic acids[50], o-benzamidophenoxy and o-benzamidophenyl acetic acids[51] have also been claimed.

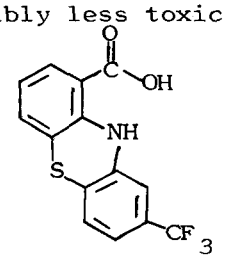
As with indomethacin analogs, the in vivo biological activity of several α-methylphenylacetic acids is invariably associated with the dextro rotatory isomer of the sinister absolute configuration.[47] Thus the stereo-specificity of the α-carbon atom of these anti-inflammatory agents appears to be identical with that of plant growth hormones.

Fenamates - The antirheumatic efficacy of both mefenamic acid and flufenamic acid (III) have been demonstrated in the clinic.[52,53] The equipotency dose was estimated as aspirin 2.4 g. mefenamic acid 1.7g., flufenamic acid 0.67 g. and phenylbutazone 0.33 g., rather at variance with their activities in animals. No outstanding differences between the side-effects of the four drugs were reported in this study. Clinical data on the more potent congener, meclofenamic acid (CI 583, XIII) has not been published.

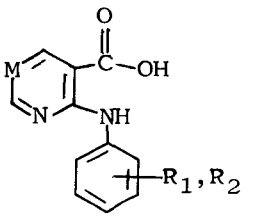


XIII

Interest in close analogs of fenamates is illustrated by 8-trifluoromethyl phenothiazine-1-carboxylic acid (XIV)[54] (an S-bridged analog) and the acetic acid homologs.[55] Heterocyclic analogs are exemplified by several pyrimidine (XV)[56] and pyridine (XVI)[57] isosters. Replacement of the carboxyl group by a tetrazole[58] resulted in a slightly less active but possibly less toxic analog. It is noteworthy that the optimal activity



XIV

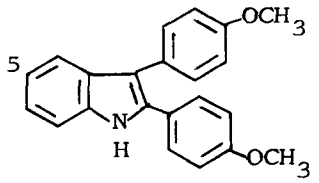


XV M= N

XVI M= C

enhancing groups of these analogs apparently are considered to be identical with those in fenamates, i.e., 2,6-diCl-3-Me, 3-$CF_3$ and 2, 3-diMe, originally selected by the Parke-Davis group.

Indoxole - In the chemical study of indoxole (XVII)[59] and its analogs the minimum structure requirement for significant activity was defined as 2,3-diphenyl indole. The potency enhancing effect of two p-methoxy groups was specific and additive. Substitutions at $C_5$ of the indole ring (a sensitive position in the indomethacin series) by Cl, F and $CH_3$ had little influence on the activity, whereas $N_1$-alkylation and acylation were detrimental. The highly conjugated system has strong U.V. absorptions at ca. 250 and 310 mμ suggesting that the two anisyl groups, like the aryl groups in fenamates and phenylbutazone, are not coplanar. Biological activity in animals was related to the log of the average serum level which is largely determined by the solubilizing vehicles used in formulation.[20,60] Indoxole was modestly active in crystal-induced synovitis in man at 2.4 g/day.[61]

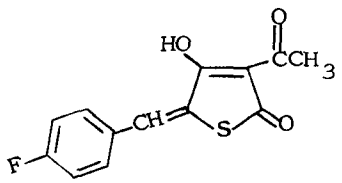


XVII

Sulfhydryl Compounds - The anti-rheumatic activity of penicillamine (I) has been attributed by Lorber[12] to its ability to dissociate abnormal macroglobulin and to restore serum sulfhydryl levels in vivo, either through sulfhydryl-disulfide interchange or chelation of divalent metal ions like copper which catalyze the oxidation of sulfhydryl groups. Penicillamine, cysteine and other thiol compounds are capable of solubilizing collagens in vitro[62] as well as in vivo.[63] Inhibition of kallikrein and foot-edema in guinea pigs by these compounds has also been noted.[64]

Immune Suppressive Agents - Satisfactory clinical results have been obtained in the treatment of rheumatoid arthritis with an immune suppressive agent cyclophosphamide[65], but wider application must await the availability of a safer and less toxic agent.

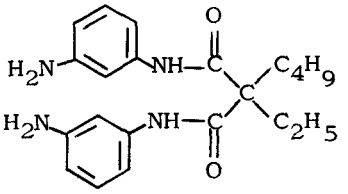
Following the discovery of a novel antipolyarthritic agent I.C.I. 43,823[17], a thiophene derivative, I.C.I. 47,776 (XVIII), was described as a new immune suppressive agent capable of inhibiting the development of polyarthritic lesions, antibody response and other autoimmune syndromes in rats. The inhibition was attributed to interference with generation of high energy phosphate esters necessary for protein and nucleic acid synthesis. These potent cytotoxic agents are valuable laboratory tools



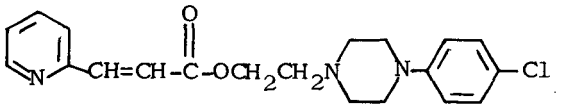
XVIII

but of limited clinical applications.

Miscellaneous - Two novel agents reported to possess antiinflammatory, antipyretic and analgesic activities nearly comparable to phenylbutazone are N,N-Di(m-aminophenyl)butyl-ethylmalonamide (Gy-97, XIX)[66] and 1-(4-chlorophenyl)-4-{2-[3-(2-pyridyl)acryloxy]ethyl} piperazine (DA 1529 XX).[67] Gy-97 also has anti-histaminic and anti-



XIX



XX

anaphylactic properties, whereas DA 1529 possesses mild CNS depressant properties.

The biochemical properties of chloroquine and related antimalarials have been examined further. In addition to its inhibition of the biosynthesis of sulfated mucopolysaccharides, chloroquine inhibits irreversibly an autolytic enzyme from bovine cartilage and a rat skin collagenase (at 10 mM).[68] Like hydrocortisone, it inhibits chemotaxis of leukocytes and, to a lesser extent, the phagocytosis process.[13] It also stabilizes lysosomal membranes in vitro. Potential "anti-degenerative" activity is clearly suggested by these properties, but unfortunately the well-known retinopathic effect is further complicated by a delayed symptom.[69]

Proceedings of an international conference on dimethylsulfoxide have been summarized.[70] The FDA restriction of DMSO has been modified to allow limited investigations of this unique agent. One of its metabolites, the crystalline dimethylsulfone, also possesses a weak antiinflammatory activity.[71] On the other hand the phenyl derivatives, benzyl methyl sulfones and sulfoxides[72], have been found to be more potent but short acting anti-edema and analgesic agents.

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Section V - Topics in Biology
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Chapter 22. Molecular Aspects of Drug-Receptor Interactions.
Barry M. Bloom, Chas. Pfizer & Co., Inc., Groton, Connecticut

> "Membrane biochemistry occupies a central position in modern biology, second in importance, perhaps, only to biochemical genetics. Replication and organization are the significant differences between living and non-living catalytic systems, and cellular organization is a function of membranes." - Edward D. Korn[1]

## Membrane Biology

There is a growing realization among drug research scientists that many receptor systems of major interest are located in the plasma membranes of cells. Sites responsive to acetylcholine, epinephrine, histamine, serotonin, peptide hormones such as insulin and oxytocin, and the plasma kinins are almost certainly among this group. These receptor systems usually remain fully responsive only as long as the structural integrity of their highly architectured residence is maintained, and therein lies the main stumbling block to their direct study along lines successful with enzyme systems. Nevertheless, the present decade has witnessed remarkable progress in the isolation and analysis of various plasma membrane systems, and new knowledge has been amassed at an impressive pace. It seemed, therefore, particularly timely this year to review those aspects of the field of membrane biochemistry and ultrastructure that appear most pertinent to the study of drug receptor systems.

The Repeating Unit Concept of Biological Membranes - An important concept has emerged in recent months that deviates importantly from earlier views on the nature of biological membrane systems. The theory, as put forward by Benson[2] and by Green and his colleagues[3-6], suggests that biological membranes are vesicular or tubular systems built up of nesting, lipoprotein repeating units one layer thick. This viewpoint, along with a considerable amount of compelling evidence that supports it, has been brilliantly summarized in several papers. One chapter in a new book by Green and Goldberger[6] provides an outstanding introduction to the field of membrane biology, viewed from this new perspective.

Judging from the evidence at hand, it would appear that the repeating unit visualization seems destined to replace the classical Danielli-Davson model of plasma membrane ultrastructure that has dominated thinking on the subject ever since it was first proposed in 1935. In the classical model, as well as its more contemporary variant, the Robertson unit membrane hypothesis, membranes are seen to consist of a bimolecular leaflet of phospholipid sandwiched between two layers of globular protein. The non-polar fatty acid chains of the phospholipid are oriented inward, perpendicular to the plane of the membrane system. The polar groups of the phospholipid at the external surface of the bimolecular leaflet are presumed to bond electrostatically to the protein, affording

two essentially separate but continuous phases. Excellent reviews and critiques of these earlier hypotheses are available[1,7].

Because of the potentially great heuristic value of knowledge about membrane structure, the following three sections are devoted to a summary of the repeating unit concept as it appears most relevant to the study of receptor systems. Mention is made of the molecular nature of the repeating units, the types of cohesive and repulsive forces that appear to be important in membrane systems, and discernible relationships between form and function. The probable mode of membrane biosynthesis is reviewed as a likely source of fundamental insights.

Membrane Formation - The formation of a biological membrane, according to the repeating unit viewpoint, begins with the genetically-controlled synthesis of the individual component proteins and phospholipids. These interact through specific hydrophobic bond formation to afford one repeating unit or macromolecular lipoprotein complex. It assumes the conformation thermodynamically most stable under physiological conditions. The repeating units then aggregate in two dimensions with similar (or different but compatible) particles to form the membrane continuum. This latter process occurs spontaneously in the proper environment and requires no "informational" molecules. The resulting structure is dynamic in character. A constant input of metabolic energy is required to maintain its structural integrity, and it undergoes conformational changes quite readily under the influence of various stimuli.

In contrast to classical membrane theory, the repeating unit concept is clearly compatible with the fundamentals of molecular genetics, in that organization and function are controlled through the mechanisms of protein synthesis. Genetically-determined amino acid sequences are seen to afford protein tertiary structures characterized by a hydrophobic interior that could facilitate specific associations with the lipid chains of particular phospholipid components. It is an established fact that the phospholipids of mammalian tissue are highly specific as to their fatty acid content (another point of genetic control), so that one would anticipate a high order of influence for the protein amino acid sequence on which lipids are most stably bound to them, as well as where and how they are bound. This, in turn, would largely determine the tertiary structure of the protein-phospholipid macromolecular complex, including such key features as the distribution of polar functionality on the membrane surface.

The phospholipid component appears to determine the preferred mode of combination of repeating units with one another. Lipid-free particles polymerize to three-dimensional aggregates, which are essentially bulk phases devoid of enzymatic activity. Reintroduction of phospholipid restricts the repeating units to "side to side" interactions involving predominantly hydrophobic protein-to-protein bonds. This type of interaction affords an enzymatically-active membrane continuum. It has been concluded, therefore, that the essentiality of phospholipid for normal enzymatic function in such systems reflects their ability to "direct" membrane formation rather than any specific chemical effect exerted directly on the enzyme[5].

Repeating Unit Composition - The repeating units that have been detected in various types of cell membranes appear to be made up of so-called base pieces, which are the sub-units essential to membrane formation, and detachable portions (headpieces and stalks) which contain enzymatic activity, but are not essential to the membrane continuum per se. The headpiece and stalk can be detached through procedures such as sonication or detergent action, without disruption of the membrane. The repeating units themselves can also be disassociated by detergent action. Upon removal of the disassociating influence, the units will spontaneously reaggregate to form vesicular membranes. Going one step further, solvent extraction of lipid from repeating units removes their ability to form membranes, but this can be restored by reintroduction of the extracted lipid. The tendency to form membranes would, therefore, seem to be an expression of the intrinsic chemical properties of these lipoprotein repeating units.

The molecular weight of repeating units appears to range from 50,000 to over a million. The particles from inner mitochondrial membrane, for example, have a size (MW about 1.3 million; diameter about 150Å) that corresponds to about 600 phospholipid molecules (MW = 800) and 30 protein molecules (MW = 25,000). On the assumption that the base piece constitutes about 40% of the entire repeating unit, Green and Goldberger[6] estimate that it would contain about six each of structural and catalytic proteins, along with about 240 phospholipid molecules. Membranes commonly appear to possess structural and catalytic proteins in about a 1:1 ratio.

Membrane Function - The primary functional properties manifested by plasma membranes include a selective permeability toward ions and molecules, the ability to translocate such species at the expense of metabolic energy, and highly specialized enzymatic activities characteristic of the function of the parent tissue. They also often contain glycolytic enzymes to help meet energy needs. Determining the location of the enzymatic activity responsible for any of these functions is a complicated problem in itself. Most systems are characterized by a multiplicity of membranes, single membranes being the exception. Location thus becomes a question of which membrane, which species of repeating unit, and which sector of the repeating unit is involved.

Repeating units of varying composition can apparently be sufficiently complimentary to form a given membrane, and enzymatic properties are often parcelled out in blocs to different species of repeating units. For example, it is felt that the inner membrane of the mitochondrion may be comprised of as many as ten species of elementary particles, each chemically and enzymatically different, with electron transfer involving a set of four of these.

It is intriguing to speculate whether this same kind of ultrastructural differentiation (different repeating units) underlies the recognized ability of a single type of smooth muscle cell, for example, to respond to stimulation by a number of different hormones and humoral substances, each in a chemically precise but different way. Hokin[8] has already suggested the existence of "membrane patches of active liproprotein

specialized to carry out various functions."

Permeability characteristics toward ions and polar molecules, which vary greatly among membranes, presumably reflect the tightness of fit among repeating units, as well as the nature of the functionality lining the potential pores or channels. Permeability modification, by metal ions for example, is attributable to their ability to change the size, shape and volume of repeating units, presumably by interacting with phospholipid anionic centers. This can induce phase transitions[9] which profoundly alter the nature of the membrane. In fact, the action of metal ions can entirely transform the shape of the organelle or cell involved.

Selected References on Membrane Biology - A number of other important papers and books on membrane biology have been published recently. Kavanau[10] has written two provocative volumes in which he views the biological membrane as a dynamic and highly labile entity, shifting between different substructural states with different phases of function. The proceedings of symposia on membrane biology have been reviewed[11] and published[12,13]. The complexities of membrane phospholipids have been reviewed in some detail[14-17], and their role in membrane function analyzed, especially by the Hokins[18]. Wolfe[19] has provided a useful review of cell membrane constituents as they relate to transport mechanisms. Kennedy and his colleagues[20-22] have conducted well-designed experiments with the β-galactoside permease system in E. coli. Other noteworthy papers discuss the properties and stabilization of lysosomal membranes[23] and the similar effects of phospholipase C (a phospholipid hydrolyzing enzyme) and insulin on glucose transport in isolated fat cells[24].

Two recent studies[25,26], on the molecular nature of cell membrane protein offer an excellent illustration of the power of modern instrumental techniques in this field of biology. They provide evidence that supports a model of membrane structure closely akin to the repeating unit visualization, through the use of ORD and CD coupled with refined methods of membrane fractionation. Additional information of importance was obtained on the degree of similarity among membrane structural proteins from different types of cells, the proportion of membrane protein that seems to assume a helical conformation, and the hydrophobic nature of the membrane interior. These same two papers and others[27-32] serve as excellent leading references to methods of membrane isolation and fractionation. IR[33] and NMR[34] studies on membrane fractions have been performed with less rewarding results than the ORD and CD efforts cited.

Changeaux and his colleagues[35] have analyzed the amplifying properties of repeating unit membranes as they respond to ligand binding, from a viewpoint that reflects previous association with Monod[36] in the field of regulatory enzyme mechanisms. The result is a generalized mathematical model of the so-called cooperative properties of membranes. In effect, this constitutes the first receptor theory specifically related to the new concept of membrane structure, in that it emphasizes the relationship between the conformational transitions undergone by repeating units and ligand (drug) binding. One prediction of the model is that the concept

of "intrinsic activity" of a drug, as introduced by Ariëns, is an unnecessary one.

## Receptor Systems

The proceedings of a recent conference on adrenergic blocking drugs[37] contain a wealth of material. Belleau[37] provides a provocative stereochemical model for the adrenergic α-receptor based on the idea of catalysis of ATP cleavage to ADP by α-agonists, as proposed recently in the "dynamic receptor" hypothesis[38,39]. Certain of the structural speculations offered are clearly influenced by his assumption that the membrane cation transport ATPase system is involved. This is unfortunate, since the primary influence of α-agonists on cation transport (rapid K+ efflux) is just the opposite of the result to be expected from stimulation of that system. The ability of norepinephrine to stimulate the rate of a different phosphate ester-hydrolyzing enzyme has recently been demonstrated in a synaptic membrane fraction from rat brain homogenates[40].

Robison, Butcher and Sutherland[37] speculate that both α- and β-adrenergic receptor mechanisms are related to adenyl cyclase. Criticism of this hypothesis is summarized by Cotten[37].

Three different laboratories have reported studies of the adrenergic α-receptor employing labeled, insurmountable antagonists of the β-haloalkylamine type, which bind covalently to an essential component of this system. The carefully-designed experiments of Triggle and his colleagues[41,42] show quite clearly that the usefulness of this type of adrenergic blocking agent for receptor "structure" studies is severely limited by their low tissue specificity. For example, uptake of $H^3$-labeled N-(2-bromoethyl)-N-ethyl-N-1-naphthylmethylamine by α-receptor-containing tissue proves to be a linear function of the bath concentration of drug, even at concentrations well beyond the amount required to effect complete pharmacological blockade. It is intriguing to speculate whether this experimental approach might prove more fruitful when applied to an appropriate membrane fraction rich in receptor material. This question seems likely to receive attention as soon as the necessary isolation and purification techniques are mastered in laboratories conducting receptor research.

Despite experimental limitations, these studies with β-haloalkylamine antagonists have led to certain conclusions regarding the concentration of α-receptors and the significance of "spare receptors" in rabbit aortic strips. Lewis and Miller[43,44] have analyzed the same problems, using $H^3$-phenoxybenzamine in rat seminal vesicle preparations.

Both of these groups and another from Manitoba[45] have offered criticism of certain of the data and conclusions from an earlier study by Dikstein and Sulman[46], especially the suggestion that alkylation by Dibenamine involves a cephalin-like fraction of the α-adrenergic receptor system.

Studies on isolated guinea pig vas deferens tissue[47] have been interpreted as showing that trypsin can reverse an insurmountable blockade

of adrenergic α-receptors established with a β-haloalkylamine. The authors conclude that the anionic site of the receptor is a carboxyl group derived from arginine or lysine. However, attempts to repeat these experiments elsewhere[41] have served to demonstrate that the increased responsiveness of alkylated tissue toward α-agonists, observed following incubation with trypsin, also occurs with non-alkylated (untreated) tissue. Furthermore, proteolytic enzymes unrelated to trypsin in their action are also capable of reversing the blockade, suggesting that the tissue modification involved in restoring sensitivity is not directly related to the drug-receptor complex.

Gillespie[48,49] has employed fluorescence techniques to show that smooth muscle membrane can bind norepinephrine, and that this binding can be prevented by prior treatment with the α-adrenergic blocking agent, phenoxybenzamine.

A recent series of pharmacological studies has analyzed certain steric aspects of adrenergic drug structures[50,51].

Biel and Lum[52] and Ariëns[37] have reviewed the rapidly expanding field of β-adrenergic receptor blocking agents, emphasizing their pharmacology and important structure-activity relationships.

Pratesi, Lilla and Grana[53] have analyzed a group of nuclear-substituted N-isopropylphenethanolamines for the relationship between their properties as β-adrenergic blocking agents and their lipophilic character. A linear correlation was observed between lipophilicity($\pi$ in the Hantsch sense) and non-specific spasmolytic activity, but specific, competitive antagonist activity at β-receptors proved to be a quadratic function of $\pi$.

Wenke has done a scholarly analysis of the effects of catecholamines, their analogs, and various blocking agents on the adrenergic receptors regulating lipid mobilization in adipose tissue. His reviews of the subject[37,54], as well as experimental studies published recently with several colleagues[55], go far to dispel existing confusion about the nature of the adrenergic receptor mechanisms that serve to regulate lipolysis.

The relationship between an observed physiological response and the receptor event that triggers it often introduces complexities that are a source of serious confusion to those studying mechanism at the receptor level. Any knowledge that contributes to a better understanding of such relationships is worth having. The so-called cascade reaction, wherein a weak initial stimulus undergoes a series of amplification reactions to provide a strong ultimate response, has been clearly explained, using epinephrine-induced glycogenolysis as an example[56]. Visual excitation and the blood coagulation mechanism are other recognized examples of cascade processes[57].

A generalized analysis of the stimulus-response-recovery cycle and its relationship to receptors has been offered by Dikstein and Sulman[58].

A preliminary attempt has been made to label the muscarinic

cholinergic receptor, along lines similar to the α-adrenergic receptor studies already mentioned[59]. $H^3$-Dibenamine was employed, with atropine as protecting agent. Future experiments will doubtless employ the new alkylating species, N-2-chloroethyl-N-methyl-2-aminoethyl benzilate, recently described by Gill and Rang[60]. This compound, which is analogous in structure to benzilylcholine, is a highly specific, long-lasting antagonist which effectively inhibits the muscarinic actions of exogenous acetylcholine. Benzilylcholine and hyoscine protect against the pharmacological blockade it induces.

Ariens[61,62] has reported finding potent anticholinergic activity among benzilylcholine analogs devoid of any cationic head (amine moiety). The concept of an interaction of a competitive antagonist with a receptor area "additional" to that occupied by the corresponding agonist would appear to have important implications to the analysis of receptor mechanisms at the molecular level.

Elucidation of the molecular nature of another cholinergic receptor system, the enzyme acetylcholinesterase, is also being attempted through the use of specific, irreversible inhibitors. Belleau and Tani[63] have recently demonstrated that N,N-dimethyl-2-chloro-2 phenylethylamine inactivates erythrocyte acetylcholinesterase when acetylcholine is the substrate. Other workers[64] have provided evidence that the action of this compound involves alkylation at or near the anionic site of the enzyme, rather then at the esteratic site. Future studies of this system may well be conducted with pure, crystalline enzyme, if a recent report[65] of the crystallization of acetylcholinesterase from eel electric tissue can be confirmed.

Changeaux[66] has studied the effect of changing ionic strength on an elasmobranch acetylcholinesterase preparation. In solutions of low salt concentration, the enzyme tends to form rapidly sedimenting aggregates and displays a high affinity for its substrate as well as for inhibitors like decamethonium. Curare-like drugs, which are ordinarily rather poor inhibitors of the enzyme, actually bind to it rather strongly under these conditions. The results are analyzed from a viewpoint that assumes the existence of two conformational states of the enzyme in reversible equilibrium with one another.

Mapping studies of acetylcholinesterase have been conducted in the intact electroplax with a series of inhibitory 1-methyl-hydroxyquinolinium compounds[67]. The presence of a hydroxyl group located about 5Å. from the quaternary nitrogen (as in 1-methyl-7-hydroxyquinolinium iodide), increases the inhibitory strength more than a hundredfold.

Spectral studies of acetylcholine in solution[68] support the conclusion that the influence of the quaternary nitrogen on the reactivity of the carbonyl group is inductive in nature and is not transmitted directly through space.

Cuthbert[69] has made a careful study of the effect of various hydrolytic enzymes on acetylcholine-responsive systems in guinea pig

taenia coli.

Hydrolytic enzymes have also proven useful in locating certain steroid hormone receptor systems. Fanestil and Edelman[70] have provided evidence that aldosterone receptor sites are located in the nuclei of kidney cells, by showing that the action of trypsin or a proteinase from S. griseus decreases the ability of these nuclear sites to bind $H^3$-aldosterone. The same principle has been employed[71] to demonstrate the nuclear-myofibrillar site of estrogenic hormone receptors in the immature rat uterus, bound $H^3$-estradiol being released in this instance.

Offermeier and Ariëns[72], in an extensive and systematic study of the serotonin receptors in rat fundus and calf tracheal muscle, were able to confirm the experimental observations of Woolley and Gommi regarding the effects of sialidase and EDTA on smooth muscle sensitivity towards serotonin. However, they strongly criticized the earlier conclusion that a particular ganglioside is the serotonin receptor. Their work reveals that the observed effects are mainly attributable to EDTA alone, and are probably the result of calcium chelation.

Ash and Schild[73] have made a systematic analysis of the various types of histamine receptors, using several histamine analogs. From studies of gastric secretion, inhibition of uterine stimulation with carbachol, and stimulation of ileal smooth muscle, they conclude that there are at least two classes of histamine receptors.

Leading concepts of the nature of the analgesic receptor, as well as pertinent stereochemical studies of narcotic analgesics, have been reviewed by Portoghese[74]. Differing modes of drug-receptor association and the phenomenon of induced fit are favored as possible explanations for some of the complexities that characterize structure-activity relationships in this field.

Two symposia[75,76] dealing with water and its structural significance in biological systems offer provocative reading for those interested in receptor mechanisms.

## Instrumental Methods

The elucidation of macromolecular structures, and the investigation of how smaller molecules such as substrates bind to these substances, continues to be successfully accomplished through the use of modern physical techniques. It seems inevitable that these methods will make important contributions to future studies of receptor systems. The egg-white lysozyme story, involving the first successful elucidation of the three-dimensional structure of an enzyme by x-ray crystallographic analysis, has been well told by Phillips[77,78]. NMR spectroscopic studies[79] of the egg-white enzyme have revealed perturbations of the amide group in certain N-acetamido sugars bound as substrates.

In general, x-ray structural studies of biochemical interest[80] are being reported with increasing frequency. For example, two groups have

just described the structure of the complex enzyme, ribonuclease[81-83].

Computer-based approaches to molecular model-building[84] offer promise as potentially facile methods of gaining structural information about complex molecules of biological interest.

Circular dichroism studies of proteins and nucleic acids[85] are providing useful data on conformations in solution and their modification by various agents. Studies of the binding of N-acetylglucosamine to egg-white lysozyme[86] and of acetazolamide to human carbonic anhydrase[87] exemplify the application of this technique to the measurement of conformational changes induced in enzymes by small molecule substrates and inhibitors.

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# Chapter 23. Fate and Distribution of Drugs

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The present chapter has been written to bring the review presented last year up to date. Approximately 1100 references as listed in the Ringdoc Abstracts were examined; amongst them, 70 were selected from those reporting either new findings or biochemical transformations of structures not mentioned last year. Again, data are arranged in tabular form and include main pharmacodynamic property, chemical name or the generic name as listed in the Merck Index; structural formula (* indicates use of radioactive compound), with arrows indicating site(s) of biochemical reaction(s) described in the accompanying text. Abbreviations used: UCC, unchanged (parent) compound; (M) major; (m) minor; (tr) trace; (A) pharmacologically active; conj. for conjugated; gluc., glucuronide and sulf., sulfate, respectively.

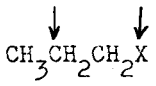
Upon examination of the reports dealing with some aspect of drug metabolism and published in 1966, we have been left with the following impressions. Firstly, one is overwhelmed by the number of studies exploring the metabolic fate of drugs and related substances. It appears that such studies are being undertaken as soon as a pharmacologically active substance begins to look like a potential candidate for clinical investigations. Often, labelled compounds are used; in the past, conclusions from absorption, distribution and excretion studies were based on data representing recoveries of total radioactivity, i.e. comprising the parent compound as well as its metabolite(s); nowadays, an attempt is usually made to recognize the main biochemical transformation(s) of a substance so as to differentiate the unchanged parent substance from its metabolite(s) in respect to distribution, life-time and excretion and, especially, to uncover whether pharmacodynamic activity is due to the parent substance or to its metabolite(s). As a consequence, data and experience gathered from such studies affect the future of a pharmacologically active substance in more than one way.

Regarding the biochemical transformations which occur on a substance in vivo, in spite of variation with species, the metabolic fate of many a compound can be anticipated with a considerable degree of confidence; this can be done because there are only few biochemical reactions involved in the process of rendering circulating substances more hydrophillic - though not necessarily less biologically active or less toxic. The paucity of these biochemical reactions coupled with the practically unlimited number of substances which are, or which can be metabolized, reflect the low degree of substrate specificity of the enzyme system(s) determining the fate of a substance in vivo; the enzyme system(s) involved in hydroxylation(s), for example, can hydroxylate a great variety of structurally quite different compounds.

At this point it is pertinent to recall the capacity of many substances to alter - enhance or block - the activity of enzyme system(s): while lasting, the change may affect not only the fate of the substance which caused it; because of the low substrate specificity mentioned above, the change may also affect the fate of any other substrate subsequently in contact with the respective enzyme system(s). It is of considerable importance to take the phenomena of enzyme inhibition and, particularly, of enzyme induction into consideration whenever the biological activity or the metabolic fate of a compound is either discussed, studied or rationalized. Appreciation of these phenomena may result in more frequent use of agents known to affect the activity of microsomal enzyme systems for a specific purpose: to alter the physiological or the pharmacodynamic effects of endogenous or exogenous substances merely by altering their metabolic fate.

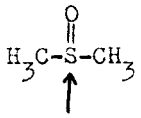
An inspiring, and likely the first, example for the therapeutic application of enzyme induction to affect the metabolic fate of an endogenous substance has recently been reported by Yaffe and his associates (71): phenobarbital treatment has been used to prevent hyperbilirubinemia: by inducing the glucuronyl transferase, enhanced glucuronide formation enabled increased bilirubin excretion and resulted in decreased serum bilirubin concentration.

## SOME METABOLIC TRANSFORMATIONS REPORTED IN 1966



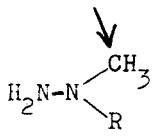
1-Halopropanes
(X=Cl, Br, I)

New metab. of propylbromide (I) in rat is n-propylmercapturic acid sulfoxide (14%), trace of S-contg. metab. suggest that S-alkyl. cystein residues formed only with (I) (1). Haloalkanes gen. metab. to mercapturic ac. and similar S-linked conj. Addl. metab. reaction is hydroxylation (2).



Dimethylsulfoxide

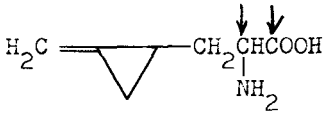
In rat, man: oxid. to $Me_2SO_2$ (rat, 15%; man, 3%); rest is UCC (3). In rabbit, UCC (35%), red. to $Me_2S$ (exhaled); oxid. to $Me_2SO_2$ (9.5%) (4). $Me_2SO_2$ (i.p.), in rat (64%) (3) and rabbits (75%) (4) excr. as UCC. $Me_2S$ (i.p.), in rabbit: UCC (exhaled); $Me_2SO$ (approx. 9%) and $Me_2SO_2$ (approx. 10%) (in urine) (4).



Methylhydrazine*
(R=H, or $CH_3$)

In rat: oxid. demethyl. to $CO_2$ for dimethyl deriv. dependent on dose; approx. 50% of monomethyl deriv. demethylated to equal quantities of $CO_2$ and $CH_4$(5).

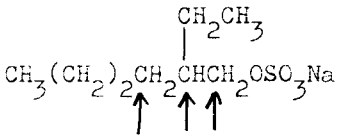
Toxic. from Blighia sapida



Hypoglycin A

In rats, rapidly oxid. In vitro, transamin. and decarbox. to methylene-cyclopropaneacetate (6), which blocks fatty acid ()$C_{10}$)oxid. in vitro; tox. of hypoglycin prob. due to this metab. (7).

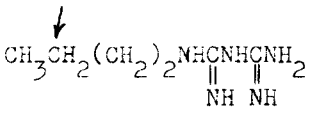
Surfactant



2-Ethylhexyl sulfate*

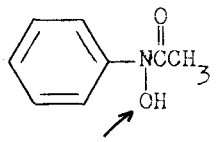
In rat: UCC (60%); 2-ethyl-2,3-dihydroxyhexanoic acid (30%); 2-ethylhexanoyl gluc. (5%); 2-ethylhexanol (1%). In rabbits, mainly UCC (8).

Hypoglycemic



1-Butylbiguanide*

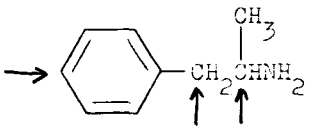
3-Hydroxy-deriv. only metab. in guinea pig, mouse, rat and rabbit. Hypoglycemic eff. proportional to UCC: high in rat, none in rabbit. In man, only UCC (9).



N-Acetyl-N-phenylhydroxylamine

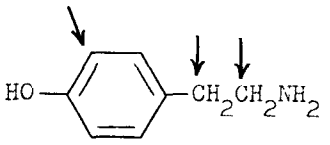
In rabbit, the N-O-C gluc. isolated and identif.; in contrast to N-gluc., the N-O-C gluc. is completely hydrol. by β-glucuronidase (10).

CNS Stimulant



d-Amphetamine*

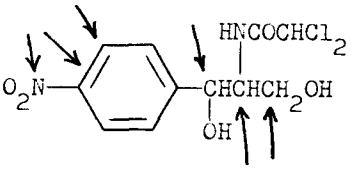
UCC, rat (m), dog (M), monkey (M) (11), man (M) (12,13), rab. (tr); p-OH and gluc., rat (M), dog (m), monkey (tr) (11), rab., man (m) (12); benzylmethylketone, rab. (m), dog (tr); phenylpropanol, rab. (m), dog (tr) (12); hippuric ac., rat (tr), dog (M), monkey (m) (11), rab., man (M) (12,13). In rat, diff. in metab. of (+) and (-) forms (12).



In rabbit: p-OH-phenylacetate major metab. Also, β-oxid., oxid. deam., m-hydrox. and m-O-methyl.; important finding: depending on sequence, octopamine (approx. 7%) and/or catecholamines are formed (14).

Tyramine*

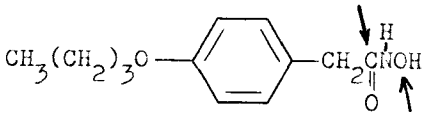
Antibiotic



In cult. medium of bact. growing on chloramphenicol as source of C, series of metabolites identified, indicating stepwise degradation to β-carboxy-cis,cis-muconic acid (15).

Chloramphenicol

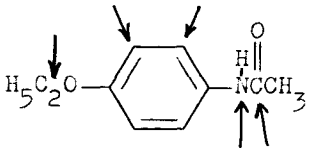
Anti-inflammatory



In rat, UCC (m); of the 11 metabolites, 4-butoxyphenylacetamide and corresp. acid identified. Rapid abs. and distrib. (rat, rabbit); after 48 hr approx. 45% in urine, 30% in faeces (UCC) (16).

4-Butoxyphenylacetyl-hydroxamic acid*

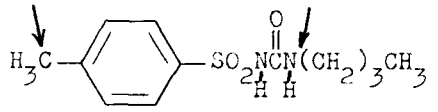
Analgesic



In man, rat: UCC (tr); O-dealkyl. and gluc. (man 28%, rat 22%), sulf. (man 8%, rat 6%); m-hydroxyl. (rat, <0.01%). o-Sulfonyloxy-metab. is new (17). 2-Hydroxyl. new metab. in cat, dog, man; also, N-OH-metab. (tr) (18).

Acetophenetidine

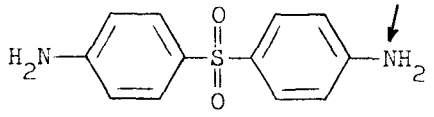
Hypoglycemic



Tolbutamide*

In man, 85% recovered as 4-$CH_2OH$- and 4-COOH metabolites. In rat, 4-$CH_2OH$ (M) with 4-$COOH_3$(tr) and N-dealk. (tr). Use of $^3$H-label clarified earlier discrepancies. In rat, rel. amts. of metabolites not changed after chronic admin. (19).

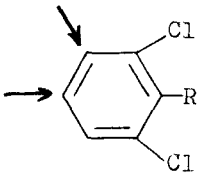
Anti-leprosy



Dapsone

In man, the sulfone well abs. and excr. (75%/24 hr), slowly metab.: UCC (up to 41%, pH-depend.); probably N-conj. (gluc.) (27%). Corresp. sulfoxide less well absorbed; oxid. to sulfone (10%) (20).

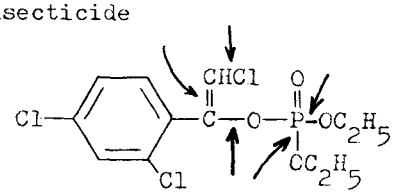
Herbicides



2,6-Dichlorobenzonitrile (R=CN)
2,6-Dichlorothiobenzamide ($R=CSNH_2$)

In rat, rab., dog: rapid abs., metab. and excr. UCC (tr); 3-OH (M), 4-OH (M) and conj. (gluc., sulf., marcapturic ac.); CN hydrol. (tr) (21,22). Fate of thiobenzamide similar, as nitrile major metab. (21). Phenolic metab. uncouple oxid. phosphoryl., may explain hepatotox. (in rab.) (23).

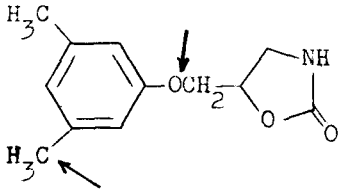
Insecticide



Chlorphenvinphos*

Completely metabolized in rat, dog; O-des-ethylation with side-chain dechlorination, dephosphorylation and side-chain oxid. to produce 6-7 metabolites (24).

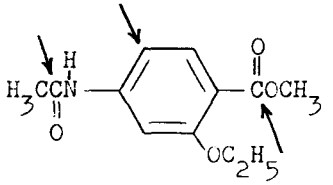
Muscle relaxant



Metaxalone

In dog, man: oxid. of 3-methyl to COOH (27%) followed by conj. (gluc.) (17%); cleavage of ether bond (m). Rapid excr. (48 hr) in rabbit (96%) and rat (71%), slow in dog (11%). Metabolism similar in rabbit and dog, different in rat (25).

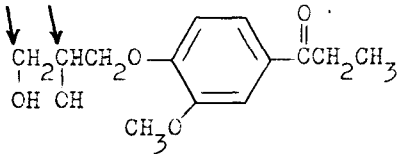
Coccidiostat



Ethopabate*

In chicken: ester hydrol. (20%), with (a) N-des-acetylation (15%), (b) N-des-acetylation, 5-hydroxyl. and conj. (sulf.) (25%); unident. conj. (25%) (26).

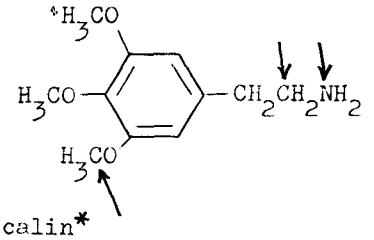
CN depressant



Meprophendiol

UCC, rat (M), rabbit (m); progressive oxid. of glycerol chain: corresp. deriv. of α-OH-propionic acid, rat (m), rabbit (M) and of acetic acid, rat (m), rabbit (tr). Both metabolites lack CN depressant act. (27).

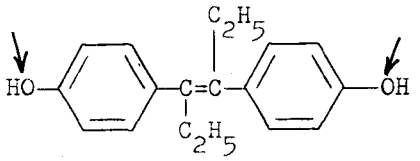
Hallucinogen



Mescalin*

In man: rapid abs., metab. and excr. (87%); UCC (55-60%); oxid. deamin. (27-30%); N-acetylation (0.1%); N-acetylation and 5-O-demeth. (5%). Pattern of metab. in cerebrosp. fl. indicates N-acetyl. more important in CNS than oxid. deamin. N-Ac-metabolite inact. in man (28).

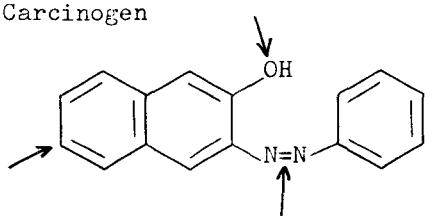
Estrogen



In rat, conj. (monogluc.) and 3 unidentified metabolites; glucuronide excreted via bile (29).

Stilbestrol*

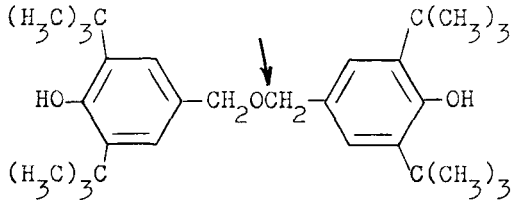
Carcinogen



In rabbit, 7 metabolites which include hydroxylation with conj. (gluc., sulf.); N-gluc; reduction and fission of azo-bond (30).

1-Phenylazo-2-naphthol

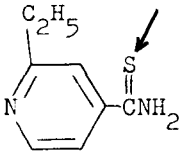
Antioxidant



In rat, dibenzylether is split with subsequent oxidation to corresp. benzoic acid (M) and its ester glucuronide (M). Intermediate aldehyde also found (m) (31).

Ionox 201*

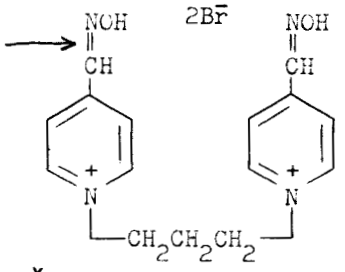
Antituberculosis



In man (32), mice, rats and dogs, ethionamide and the sulfoxide (its metab.) are interconvertible: both appear in blood, irresp. of which cpd. was given (33).

Ethioniamide

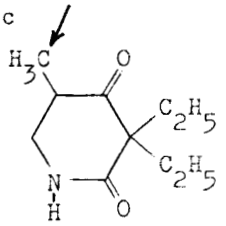
Anti-cholinesterase-inhibitor



TMB-4*

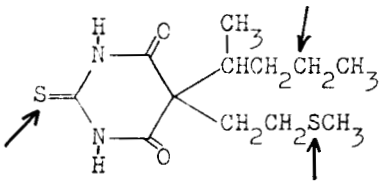
In rat (i.p.): UCC (large quant.); dehydration to mono-CN metabolite; additional metabolites detected (34).

Hypnotic



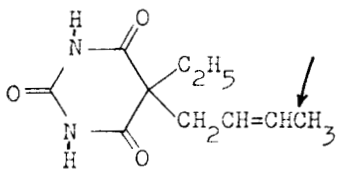
Methyprylon

In man, 6-oxo metabolite is new (35).



Methitural

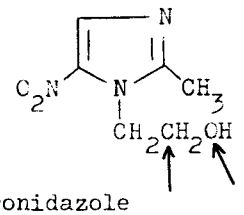
In dog, three new metabolites isolated: 3'-carboxy-, sulfoxy- and sulfono-derivatives of barbituric acid (36).



5-Ethyl-5-crotylbarbituric acid

In rat, oxidation of terminal C-at. to acid (M) of interest since usually, penultimate C-at. is oxidized. Addl. metab. found (tr) (probably unsat. alcohol) (37).

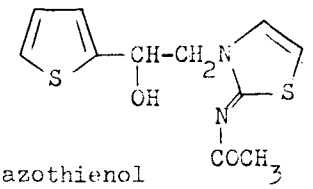
Antiprotozoa



Metronidazole

Metab. pattern similar in man and dog: UCC ()60%); oxid. of $CH_2OH$ to COOH ()25%); ether gluc. of $CH_2OH$ ()5%); no reduction of $NO_2$ detected (38).

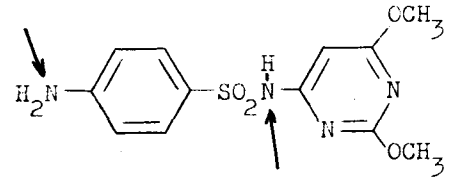
Anthelmintic



Thiazothienol

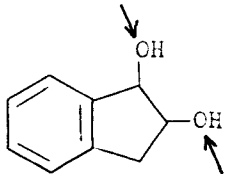
Major metabolite (in faeces) is 5,6-dihydro-6-(2-thienyl)imidazo[2,1-b]thiazole, more potent antihelmintic than parent drug. Finding led to synthesis of tetramisole (corresp. 6-phenyl-thiazoline deriv.), potent broad-spectrum antihelmintic (39,40).

Antibacterial



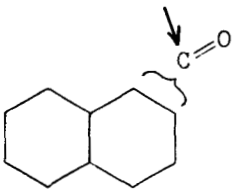
Sulfadimethoxine

Metab. of $CH_3O$-subst. 6-sulphanilamidopyrimidines depends on pos. of $CH_3O$; e.g. (a) 2,4,5-$(OCH_3)_3$: N'-conj. (gluc.), monkey (M), rat, rab. (m); $N^4$-acetyl., monkey (m), rat, rab. (M); (b) 2,5-$(OCH_3)_2$: UCC (M); $N^4$-acetyl.(tr); $N^4$-conj. (gluc., sulf.) (tr) (41).



Indane-1,2-diol

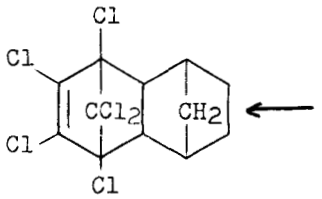
Metab. studies with the cis- and trans- form in the rat suggest that interconversion occurs via oxidation to ketone and reduction back to alcohol (42).



Decalone, 1 or 2

The metabolism of the isomeric forms of 1- and 2-decalone was investig. in the rabbit: reduction to alcohol (10-50%) and conj. (gluc.) (20-50%) (M) (43).

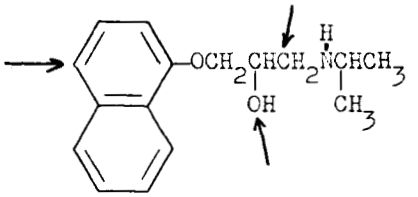
Insecticide



Dihydroaldrin

In housefly and pig microsomes: mixt. of endo- and exo-6-OH-metab. Inhib. of hydroxylation by sesoxane, may account for synergism in vivo. In vitro, dihydroaldrin inhibits competitively the epoxidation of aldrin (44).

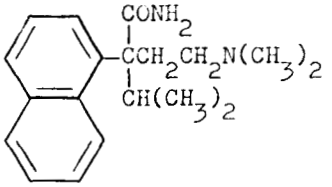
Adrenergic β-blocker



Propranolol

In mice, rats, guinea pig and man: UCC (tr); conj. (gluc.); 4'-hydroxylation followed by conj. (gluc.) (M); oxidative deamination (approx. 30%) (45).

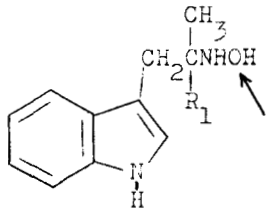
Anti-inflammatory



Naphthypramide

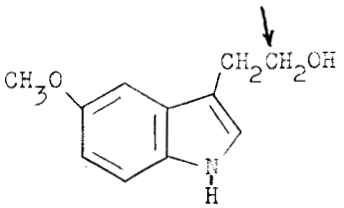
Not metabolized in rat, rabbit, man. In man, 50% excr./24 hr. In rabbit, 48% undergoes tubular reabsorption (46).

Stimulants



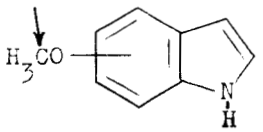
Indolylalkylhydroxylamines
($R_1$= H or $CH_3$)

Hydroxylamine moiety reduced to corresp. amine by mouse tissues. Metab. probably responsible for stimulant action (47).



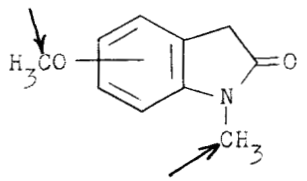
5-Methoxytryptophol*

In rat: oxid. to corresp. acid (93%); conj. (prob. with glycine) (m); hence, 5-methoxyindole-3-acetate acid in pineal tissue is probably a metabolite of 5-methoxytryptophol rather than the product of pineal hydroxyindol-O-methyltransferase (48).



Methoxyindoles

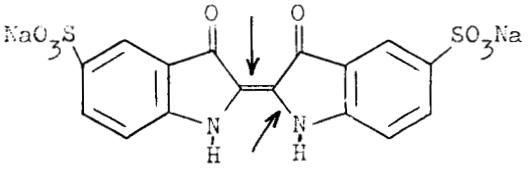
In rat, guinea pig, rabbit: O-des-methyl. of 5-$OCH_3$ and 6-$OCH_3$ derivs.; 5-hydroxyl. and partial O-des-methyl. of 4-$OCH_3$ and 7-$OCH_3$ derivs. In vitro O-des-methyl. in rat liver slices, but not in microsom. prep. (49).



Methoxyoxindoles

In vitro comparison of O- and N-demethyl. and ring-hydroxyl. of 4-isomeric methoxy- and N-methyl-methoxyoxindoles by rat, guinea pig and rabbit liver microsomes. Large differences noted (50).

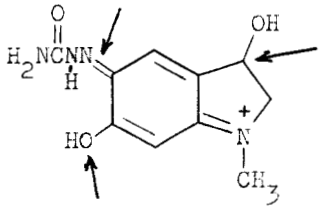
Diagnostic aid
Food colour



FD and C Blue No.2*

In rats (i.v.), bile (10%/30 min), urine (65%/6 hr): UCC (45%), isatin-5-sulfon. (12%), sulfoanthranilate (6%); orally, poor abs. (3%/72 hr urine). Interpretation of kidney function test may be complicated if dye is metabolized in humans (51).

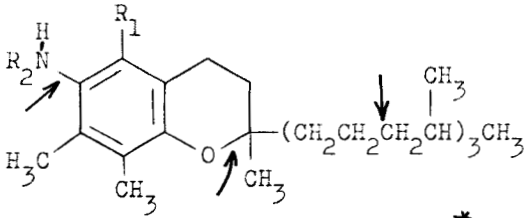
Hemostatic



Adrenochrome semicarbazone*

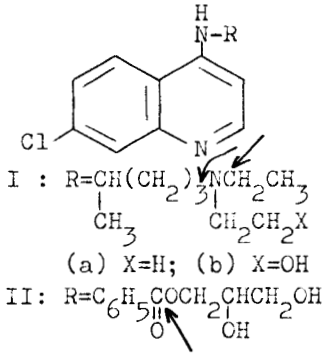
In rat, 90% UCC; three minor metabolites formed: one unidentif., other two are sulf. conj. of phenolic OH of corresp. indole derivs., with and without retention of semicarbazone (52).

Vit. E-act.



α-Tocopheramine ($R_1$=$CH_3$, $R_2$=H)*
N-Methyl-γ-tocopheramine ($R_1$=H, $R_2$=$CH_3$)*

In rats, both cpds. have similar absorption and distribution pattern. Same final metabs. as α-tocopherol, but vit. E-act. not due to metabolism to α-tocopherol (53).

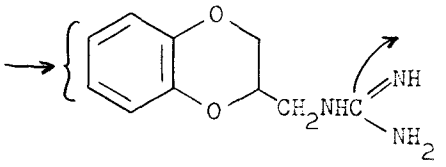


I : R=$CH(CH_2)_3NCH_2CH_3$ (with $CH_3$ and $CH_2CH_2X$)
(a) X=H; (b) X=OH
II: R=$C_6H_5COCH_2CHCH_2OH$ (with O and OH)

Chloroquine (Ia), Hydroxychloroquine (Ib): In man: UCC (60%); N-desethyl. (37%); N-bis-desethyl. (3%); oxid. deam. (tr), subsq.redn. or oxid. (monkey). Metab. of metabs. reported (54).

Glycerylaminophenaquine (II): In man: UCC; hydrol. to corresp. free acid (M); no conj. (55,56).

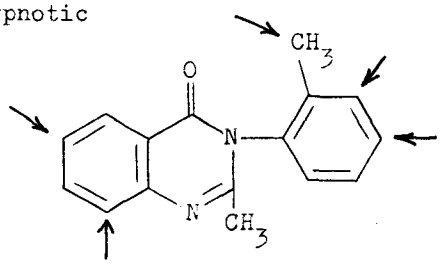
Antihypertensive



Guanoxan*

In dog: UCC (15%); transamidination to arginine (M) (5%) and guanidylacetate, the latter giving rise to creatine (10%) and creatinine (15%); hydrol. to urea (4%), but no desimidation; aromatic hydroxylation (4%), probably at C-7 (57).

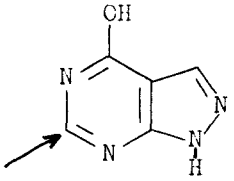
Hypnotic



Methaqualon

In rat, man: UCC; hydroxyl. and conj. (gluc.) (M) (58). In rat,rab., dog, Rhesus monkey, man: 3'-OH; 4'-OH; 6-OH; hydroxyl. of 2'-$CH_3$. In rat, rab.: 8-OH (tr); hydroxyl. of 2-$CH_3$ (not in man) (58,59). All OH-metab. are less active and less toxic (except 2'-$CH_2OH$) (59).

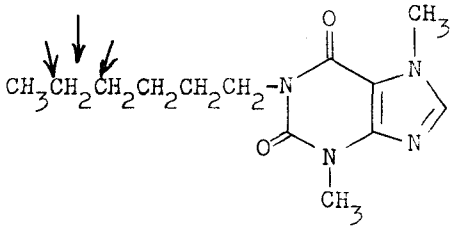
Xanthine Oxidase Inhibitor



Allopurinol*

In mice, dogs and man: 6-hydroxylation (M); small amounts of riboside may be formed (60).

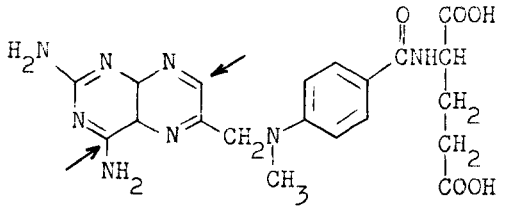
Vasodilator



1-Hexyl-3,7-dimethylxanthine (SK-7)

In rat, rabbit: UCC (tr); 5'-OH (3%) with partial conj. (gluc.), oxid. to 5-one (<1%) and hydroxyl. to 4',5'-diol (10-20%); oxid.cleavage to 3'-COOH (15-35%); diol partly conj. (gluc.). Equil. between 5'-ol and 5'-one. 1-Hexyl enhances lipid sol., hence (a) accel. abs. (oral, rectal) and metab., (b) lack of N-dealkyl. (unchanged uric ac. excr.) (61,62).

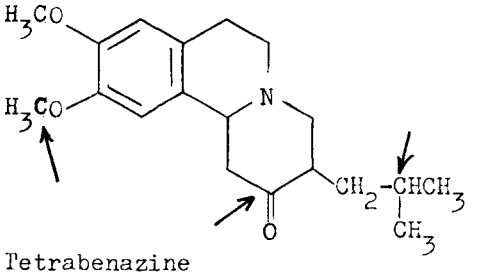
Antifolic acid



Methotrexate

In rabbits, 4,7-dihydroxy deriv. is major metab. Metab. is less toxic in mice than parent cpd. and has lower antifolic acid activity; this may explain resistance of rabbit to cytotox. of methotrexate (63).

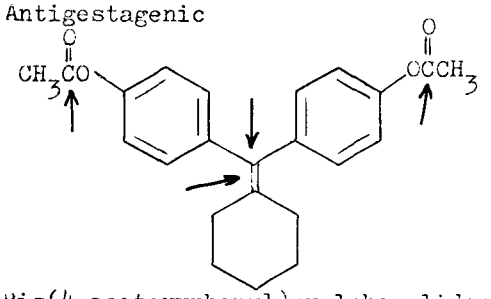
Psychotropic agent



Tetrabenazine

Nine metab. formed, similar in rab., dog and man: reduction of ketone, hydroxylation of C-2 of side chain and selective O-demethylation with conj. (gluc.) (64).

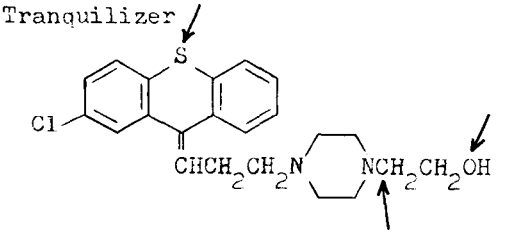
Antigestagenic



Bis(4-acetoxyphenyl)cyclohexylidene methane*

Rel. low rate of excr. in rats, rab., man. UCC (rat); des-acetyl.; bis-des-acetyl., rat (M) and conj. (gluc.); C=C cleavage, man (M) and conj. (gluc.). In rats, bulk excr. in faeces. Similar data with free di-phenol (65).

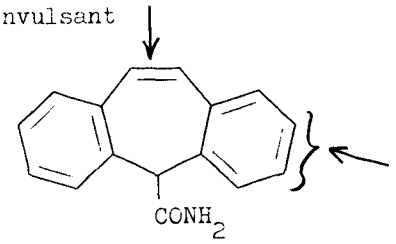
Tranquilizer



Clopenthixol

In rat, rapid abs., slow excr.; UCC (tr); conj. (gluc.)(m); oxid. to sulfoxide (M); N-dealkyl. (tr) with conj. (gluc.)(m); also unidentif., possibly phenolic metabolites found (66).

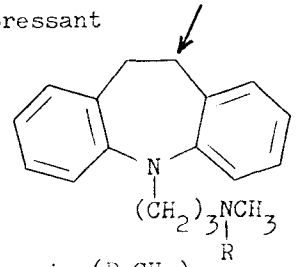
Anticonvulsant




AY-15,613*

In rat, rab., dog: ring hydroxylation (M); 10,11-bis-hydroxylation (trans) (m); conj. (gluc.) (67).

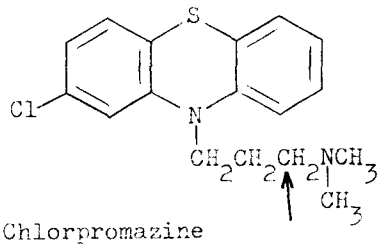
Antidepressant




I :Imipramine(R=$CH_3$)
II:Desmethylimipramine(R=H)

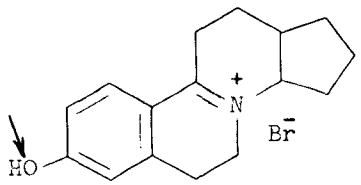
In man: following (I) or (II) 10-OH-like derivs., partly conj. (gluc.). Lack of 2,10-bis-hydroxylated metab. may indicate that aromatic (2-OH) and benzylic (10-OH) hydroxyl. represent alternative metab. pathways (68).

Tranquilizer




Chlorpromazine

In man, β-(2-chlorophenothiazinyl) propionic acid is new metabolite (0.4% of total) (69).




Quindonium bromide

Glucuronide is major metab. in mice, rats, rab., and dogs. Fluorometric assay described (70).

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## Chapter 24

# The Unknown Variable in Sensitization to Drugs: Drug or Host?

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Drug toxicity and sensitization to drugs (which might cause indistinguishable clinical symptoms) differ; toxicity has no immunological connotations while sensitization implies the presence of antibodies. Yet, unexpected toxicity as well as sensitization are likely to be results of the same 'normal' metabolic processes which are applied to any substance which is not part of our internal environment and therefore must be excreted. With insignificant exception, the effective excretion of a drug requires its biotransformation. Deamination, sulphoxidation, O- and N- dealkylation, hydroxylation of ring structures, oxidation of side chains produce a wide spectrum of metabolites.[1,2] Some of these metabolites are innocuous (as intended), some are toxic, some are neither innocuous nor toxic, but are sufficiently reactive to sensitize, i.e. to form co-valent bonds with macromolecular components of the host and to render them antigenic. It is with the formation of such antigenic determinants, 'haptens', that these reflections are concerned. Since the discussion refers, necessarily, to concepts which have been held for a number of years, the bibliography covers more than the immediate past.

Extensive in vitro studies have established that metabolites must meet certain criteria to qualify as 'haptens'. Davies, for instance, listed certain groupings which can be expected to conjugate with protein e.g. $-NH_2$ ($-NO_2$, $-N{=}N-$), $-CONH_2$, $-NHOH$, $-COOH$, $-OH$ and quinones[3], and the list has lengthened in the intervening years. In spite of our in vitro understanding, however, the incidence of clinical sensitization seems to be without rhyme or reason.

Drugs are absorbed, transported, metabolized and excreted; sensitization must occur along the way. Information about physical chemistry and biological fate of drugs is generally available. We have abundant data, for example, about solubility, ionization, half-life, protein binding, metabolic handling and mode of excretion of sulfonamides[4,5,6]; moreover, there is no doubt that sulfonamides are able to sensitize.[7]

Considering our familiarity with the pharmacological behavior of different sulfonamides, it is frustrating, indeed, to concede defeat; no correlation appears to exist between their structural or functional characteristics and their tendency to become antigenic determinants. Table I - which has been compiled and averaged from multiple sources - presents biochemical and metabolic studies of various sulfonamides and notes, for each, the reported incidence of sensitization. We have been unable to find a common denominator. Even so, it is instructive to take a critical look at certain hypotheses which have been advanced.

A short half-life, for instance, does not prevent sensitization since sulfanilamide (which is readily absorbed and excreted) and sulfathiazole (which has the shortest half-life of all sulfonamides) produce the largest number of reactions. On the surface, it seems difficult to reconcile this fact with Lehr's view that the incidence of sensitization depends on the amount and concentration of the sulfa drug to which patients are exposed but we shall return to a possible explanation of this discrepancy later on.

Several authors have speculated on the role of plasma proteins in the processing and final disposal of drugs[8], and while it has been suggested that conjugation of drugs or of their metabolites with plasma proteins might produce antigenic compounds, this tempting hypothesis has never been verified. On theoretical grounds, $N^4$-acetylated or $N^1$-conjugated sulfa derivatives, even if bound to albumin, should not be expected to become antigenic determinants. Table I supports our belief that protein binding, per se, does not explain sensitization; some sulfonamides which, like sulfisoxazole, are readily bound to albumin, cause few allergic reactions. Parker has stated that reversible binding of a simple chemical to protein, regardless of degree, is not related to its ability to sensitize [9], but the possibility exists, of course, that protein binding might <u>prevent</u> sensitization. Sulfathiazole, however, which probably produces the highest number of allergic symtoms, is bound to albumin at twice the rate of sulfadiazine (which is innocuous) and at seven times the rate of sulfanilamide which sensitizes a significant number of patients. By and large, we have no evidence that reversible binding of sulfa drugs to plasma albumin has either a positive or negative effect on sensitization.

The renal clearance of sulfa drugs, on the other hand, might have some bearing on their sensitizing potential, but the connection is, at best, suggestive. Sulfanilamide is excreted by glomerular filtration, but some of the newer sulfonamides are reabsorbed by the renal tubules. Experimental findings suggest that tubular handling of sulfa drugs might correlate with the ability of the drug to diffuse from an aqueous into a lipid solvent. Tubular reabsorption creates a long half-life; this is true for sulfadimethoxine and sulfamethoxypyridazine. While sulfadimethoxine and sulfamethoxypyridazine have a comparatively low incidence of 'conventional' immunological reactions, they are now known to give rise to a significant incidence of Stevens-Johnson syndrome which is thought to represent an immunological response. Research into its pathogenesis is complicated by the species specificity of the syndrome; like other phenomena which are caused by the biotransformation of drugs, it has no counterpart in experimental animals.

Binding of drugs to plasma albumin is 'normal', just as $N^4$-acetylation or $N^1$-conjugation with glucuronide must be considered 'normal' events which forestall the emergence of sensitizing metabolites. The low incidence of sensitization to drugs in general - considering the total number of potentially sensitizing compounds which are taken - must mean that most drugs and their metabolites are handled with uncanny skill and efficiency. If sensitization does take place, it might not necessarily reflect an obligatory, built-into-the-drug formation of an antigenic determinant, but the 'abnormal' and faulty handling of a potentially innocuous metabolite by a deficient host.

As a result of the growing suspicion that the major (and usually well-known) metabolites of common drugs might not be the metabolites which cause sensitization, the search for minor metabolites has been resumed with considerable vigor in recent years. Williams had shown some years ago that although sulfanilamide is predominantly excreted unchanged or as an $N^4$ - acetylated compound, a small amount is not acetylated, but is oxidized to 3-hydroxy-sulfanilamide in amounts up to 10% of the total recovered metabolites. The same holds true for sulfathiazole and sulfadiazine and, conceivably, for other sulfa derivatives.[10] Weinstein makes the interesting remark that sensitization is generally assumed to be caused by oxidized rather than by acetylated metabolites; and while he

does not give the source for this statement, there is good reason to believe that it is correct.[11] Penicillin sensitivity presents a similar case in point: after extensive studies of possible breakdown products, it has lately been demonstrated that major sensitization might be attributed to minor contaminants.[12] It is conceivable, in other words, that the formation of antigenic determinants indicates that the host has exhausted 'normal' metabolic pathways and has turned to pathways which produce 'haptens'.

'Normal' metabolic pathways in man may be inaccessable for a variety of reasons, e.g. a genetic absence of the appropriate enzymes, or their commitment elsewhere to cope with the simultaneous administration of drugs which compete for the same substrate.[13,14,15] Or, conceivably, the administration of large doses of a single drug which is ordinarily handled in a predictable fashion by microsomal enzymes, may exceed even briefly, their capacity. With this possibility in mind, Lehr's views should be reexamined since it seems quite possible that a drug which rarely forms sensitizing metabolites might do so when uncommonly large amounts are given.

It has never been established, however, that the appearance of metabolites which can form co-valent bonds with macromolecules in vitro, must be followed by sensitization in vivo. It is possible that the 'biochemical integrity' of an individual does not end with the selection of innocuous metabolic pathways, but might also have access to unidentified 'mechanisms of detoxication' which neutralize antigenic determinants before they can conjugate with homologous 'carriers'. The existence of such secondary means of protection is strongly suggested by a study which is frequently cited in support of the hypothesis that solubility is a primary prerequisite for sensitization. Sulzberger and his associates placed equivalent amounts of various sulfonamides on the base of an induced third-degree burn.[16] Sulfanilamide produced reactions in 22% of the patients, sulfathiazole (which produces the highest incidence of sensitization if administered systemically) in only 7%, sulfadiazine in only 5%, but the soluble sodium salt of sulfadiazine in 57%. It seems to us that these findings do not necessarily indict solubility as a factor in sensitization, but rather indicate that the skin lacks a protective mechanism which is present if the same drugs - regardless of solubility - are taken by mouth.

The defenses of the host are even more impressive if he is exposed to a drug which, like chloroquine, has a wide spectrum of reactivity in vitro as well as in vivo. This anti-malarial and anti-amebic drug has been recommended for the treatment of discoid and systemic lupus erythematosus, of autosensitivity to DNA, of polymorphic light eruptions, of rheumatoid arthritis, of hypercalcemia, and even of pulmonary lesions in sarcoidosis.[17] In vitro studies have demonstrated that chloroquine inhibits multiple enzyme systems including the NADH - cytochrome C reductase system, that it binds to melanin and DNA, that it stabilizes lysosomes, that it blocks the sulfhydryl-disulfide interchange, and that, to some degree, it suppresses inflammation. In view of this impressive array of biologic activities, it seems remarkable that chloroquine is a safe drug in that only a small number of patients become sensitive to it.

The intricate system which channels drugs through the various transformations involved in absorption, circulatory transport, metabolic handling and excretion must be endowed with certain safeguards which can prevent the development of antigenic determinants, or the conjugation of the antigenic determinant with a macromolecular carrier, even though the drug or its metabolites might fulfill one or more of the structural requirements for sensitization.

The adaption of man to chemical assault is quite extraordinary. It is intriguing that we are not more vulnerable than we are to the continued confrontation with new chemicals which challenge our microsomal enzymes. We have been preoccupied, rightly, with the structural potential of drugs and metabolites to induce sensitization. Possibly a more productive approach would evolve from turning our attention to the host to analyze his means for preventing sensitization when assessment of in vitro characteristics of the drug indicate that sensitization should occur.

| Drug | % of protein-binding at 0.4 molar conc'n. | $t_{50\%}$ hrs. | % excreted as * | | | % of sensitivity reactions after systemic administr. | % of sensitivity reactions after topical application |
|---|---|---|---|---|---|---|---|
| | | | unchanged | $N^4$-acetyl | $N^1$-glucuron. | | |
| Sulfanilamide | 12 | 9 | 30-80 | 20-60 | ** | 12 | 22 |
| Sulfathiazole | 77 | 4 | 30 | 20-60 | ** | 19 | 7 |
| Sulfadiazine | 45 | 17 | 60 | 30 | ** 10 | .6 | 5 |
| Sodium Sulfadiazine | | | | | | | 57 |
| Sulfadimethoxine (Madribon) | 98 | 35 | 7 | 15 | 78 | *** 3 | |
| Sulfisoxazole (Gantrisin) | 90 | 6 | 70 | 30 | | 6 | |
| Sulfamethoxypyridazine (Kynex) | 91 | 40 | 30 | 60 | 10 | *** 8 | |

Table I

Pharmacological data (approximate) and reported incidence of sensitization for some commonly used sulfonamides.

*assuming normal renal function.

**from 5-10% of the drug is oxidized and excreted as 3-hydroxysulfanilamide 3-hydroxysulfathiazole, 4'-hydroxysulfadiazine, respectively.

***the recognition that "long-acting" sulfonamides can cause Stevens-Johnson syndrome is of recent origin and exact figures of the incidence are not yet available.

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Chapter 25. Factors Affecting Adrenal Steroidogenesis

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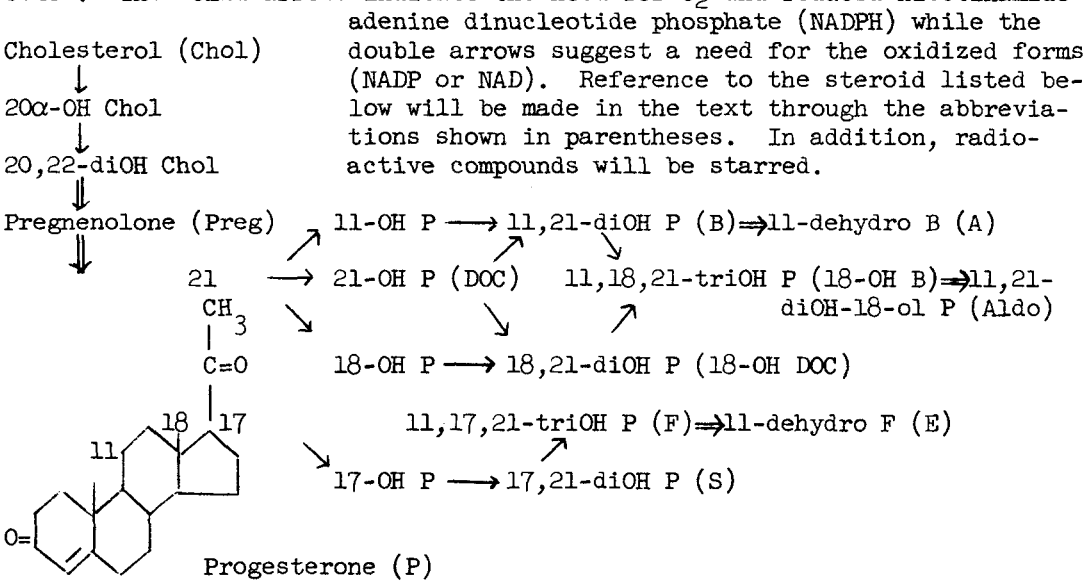
Introduction. The work to be reviewed will concern the mechanism and pathways of synthesis of adrenal corticosteroids as they may be affected by the natural hormone, ACTH, and various chemical agents. For the purpose of orientation an outline of the pathways of synthesis is included below. The solid arrows indicate the need for $O_2$ and reduced nicotinamide adenine dinucleotide phosphate (NADPH) while the double arrows suggest a need for the oxidized forms (NADP or NAD). Reference to the steroid listed below will be made in the text through the abbreviations shown in parentheses. In addition, radioactive compounds will be starred.




Pathways of Synthesis. Sulfated steroids are produced by human adrenals from both P*[1] and 20α-OH-Chol[2]. The failure of chicken adrenals to use Chol*[3] is overcome by solubilizing high specific activity material with Tween 80[4]. The failure of 20α-OH,22-keto-Chol to trap the radioactivity from Chol* mediates against its being an obligatory intermediate[5].

Mitochondrial fractions from the adrenals of several species contain enzymes necessary for steroid production. These include a solubilizable Chol side chain splitting complex[6], a non-soluble cytochrome P-450[7], 18-hydroxylase and 18-ol dehydrogenase from sheep[8] and bullfrog[9] adrenals and enzymes from the guinea pig adrenal which 11- and 21-hydroxylate, reduce ring A and remove side chain of S[10].

The 11β-hydroxylase in adrenal homogenates of the guinea pig[10] is maximally stimulated by Krebs-cycle acids in the absence of NADPH while that of the rat[11] is stimulated by both. The response in the rat varies with the Ca concentration suggesting a partial dependence on the degree of mitochondrial swelling. Minced rat adrenals convert P* to DOC and then to B and 18-OH DOC[12]. High concentrations of NADPH inhibit 21- but stim-

ulate 11β-hydroxylations of P* in rat adrenal homogenates such that 11-OH P is the major product[13].

The 18-OH DOC area on paper chromatograms may be contaminated with allotetrahydro B which is produced in smaller amounts in the presence of ACTH[14]. Another complication is that during a preincubation period, enzymes are lost from the gland which can convert Preg* to P and can hydroxylate the P in positions 17,11 and 21[15]. Glucose-6-phosphate (G6P) dehydrogenase activity is also present. Rat adrenal small particles contain a $\Delta^5$-3 ketosteroid isomerase for which NAD or NADH but not NADP or NADPH serve as activators but not electron donors or acceptors[16]. Without ACTH, the adrenal of the Mongolian gerbil converts P* to DOC, 18-OH DOC and E in vitro[17]. Animals killed with nembutal instead of ether, produce B[18] but the F and 19,21-diOH P seen in adrenal venous blood[19] is not detectable. The European eel converts P* to 17-OH P, S, F and E[20]. ACTH stimulates the steroid production of the adrenals of turtle and snake to a lesser degree than that of the bullfrog[21].

Aldo Synthesis. Bullfrog adrenal homogenates synthesizing Aldo at the high rate of 250 μg/g/hr. require NADPH, fumarate and $Mg^9$ which can be replaced by Ca or Mn. Phosphate, ATP, Na and K are not required. Rat and bovine homogenates contain an inhibitor of Aldo synthesis. The production of 18-OH B and Aldo by rat adrenal quarters increases linearly for 20 hours but, if the animals drink 1% NaCl for 8 days, production plateaus after 2 hours[22]. The accumulation of B and 18-OH DOC plateaus after 4 hours. Adrenal sections from Na-deficient rats convert B*, DOC*, P* and Preg* to Aldo and B[23]. Angiotensin II, $NH_4$ and ACTH increase production from endogenous substrates while NADP+G6P stimulate conversion of exogenous substrates as well. Angiotensin II and $NH_4$ stimulate only Aldo production while the other factors stimulate B + Aldo. These agents are believed to act between Chol and Preg. With the turtle adrenal, ACTH stimulates Aldo production 250%. Rat adrenal regeneration following enucleation or ACTH results in a depressed ability to produce Aldo and 18-OH B and an increased ability to form B and 18-OH DOC from P* and DOC*[24]. Adrenal homogenates of sheep, cow and guinea pig show a decreasing ability to convert P* or DOC* to Aldo and 18-OH B[8]. The ratio of 18-OH B/Aldo is 2 for cow and guinea pig while with sheep homogenates values of 2, 4 and 9 are seen with P*, DOC* and B* respectively. The conversion of B to Aldo requires NADPH but the presence of NADP or NAD promotes 11-dehydrogenation with the formation of 18-OH A. Sonication failed to solubilize these enzymes. They are stimulated by Ca but inhibited by Cu, Zn, Co, Hg, Fe and Mn.

Analysis of dog adrenal vein blood shows higher secretory rates for Aldo, B and F in animals on a low Na diet by factors of 8.6, 14.1 and 417, respectively[25]. Incubation of such adrenals in the absence of added substrate, however, results in a two-fold increase, in aldo, and decrease in B with no effect on F production. Because of this difference between the in vivo and in vitro responses, as well as changes in F/B ratios it is difficult to accept the proposal that Na deficiency is affecting the B to Aldo pathway. While it is shown that twice as much B* is converted to Aldo by Na deficient adrenals, the production of half the amount of B tends to increase the net specific activity of B. The amount of label in

Aldo will double even though the same number of molecules of B are converted. Thus the conversion of B to Aldo will not actually be stimulated.

In man, Na depletion is thought to act at some point prior to DOC such that the precursors are increased[26]. It is cautioned that since B production is under control of both Aldo and F stimulating factors, its measurement gives a false picture of F production. Caution is required when dexamethasone is used to shut off F associated production of B since it is reported to reduce Aldo production as well[27]. Some interactions between the F and Aldo secreting systems must exist, since feeding a high Na diet suppresses Aldo but stimulates F secretion[28].

Angiotensin II in vitro fails to stimulate Aldo production by rat[29] and bovine[30] adrenal sections. Administration for 3 days, in the rat, had the same negative effect[31]. With bovine adrenal slices, angiotensin II reduced acetate* incorporation in P and 17-OH P but increased it into all other steroids suggesting a site of action prior to the formation of Chol[30]. Thus the site of action of both Na deficiency and angiotensin II remains elusive.

While Na deficiency will increase the juxtaglomerular index (JGI) of the kidney and will result in increased Aldo secretion, division of the common bile duct also increases the JGI but does not necessarily result in ascites or an elevated Aldo secretion[32]. Surgical relief of an otherwise intractable hyperaldosteronism resulting from severe hepatic outflow obstruction is achieved by a side-to-side portacaval shunt[33].

Inhibitors of Protein and RNA Synthesis. A review of the action of ACTH[34] in 1965 points out that the obligatory requirements for RNA or protein synthesis is still open to question. The following studies do little to correct the situation. Puromycin and cycloheximide inhibit both the incorporation of amino acids* into protein and steroidogenesis by rat adrenals, in vitro[35, 36]. NADP+G6P reverses the inhibition of Aldo production but not that of leucine* incorporation[35]. These agents act similarly in vivo and in addition cause hepatic but not adrenal glycogenolysis[36]. The suggestion that glycogenolysis may, therefore, not be involved in steroidogenesis is supported by the finding that the adrenal responds even better to ACTH following a pre-incubation period that results in a loss of glycogen[36]. While cycloheximide and puromycin are thought to block the ACTH stimulated protein synthesis, no stimulation by ACTH is demonstrated. The problem is further complicated by the reported inhibition of glycine* incorporation by cyclic AMP, ACTH and NADP+G6P, all of which stimulate steroidogenesis by rat adrenal quarters[37]. The addition of theophylline, an inhibitor of cyclic AMP phosphorylase, further depresses glycine* incorporation without affecting steroid production. The suggestion is made that with theophylline, unlike with ACTH, decreased glycine* incorporation now represents decreased steroidogenesis. Fortuitously, this balances the stimulation which is expected from an inhibition of cyclic AMP phosphorylase and no change in steroid production need occur. This is unacceptable in the absence of any experimental data.

A recent study shows that adrenals of 8 hour hypophysectomized (hypoxed) rats secrete less steroids but have unchanged uridine* incorporating ability[38]. Actinomysin D inhibits uridine* and amino acid* incorporation in the absence of ACTH but has no effect on steroid secretion in the

presence of ACTH. It is concluded that the ACTH induced formation of a steroid-regulating protein (not demonstrated) does not require new RNA formation but can be directed by RNA which is stable for 8 hours. However, such data could mean that neither RNA nor protein synthesis is involved in the steroidogenic effect of ACTH.

Metyrapone (M). 2-methyl-1,2-bis-(3-pyridyl)-1-propanone, continues to be used clinically for determining the pituitary's ability to secrete ACTH. The adrenal response to the drug as measured by the secretion of F and S is found to depend on the pituitary reserve of ACTH and the degree of dose-dependent inhibition of 11β-hydroxylase[39]. The failure to include the increased secretion of DOC leads to a low value for the inhibition of the enzyme. The varied M response seen in liver disease may result from altered metabolism of the drug[40]. NADPH does not reverse the M inhibited conversion of P* to B, 18-OH B and Aldo by mouse adrenal quarters[41]. Neither 21-hydroxylation, ring A or 20-keto reduction is inhibited by M[42]. Acutely hypoxed rats secrete more blue tetrazolium reducing (BT+) steroids following M suggesting a direct stimulation of the adrenal[43]. However, rats secrete a BT- steroid, 18-OH DOC, and thus inhibition of 11- and 18-hydroxylations by M could result in an amount of BT+ DOC equal to B +18-OH DOC. While total BT+ material would increase, the total amount of steroid could be the same and a direct effect on the adrenal need not be considered.

Bovine adrenal slices produce less 11-oxysteroids and more S and DOC in the presence of M[44]. The data shows that the increase in DOC and S is much less than the decrease in 11-oxygenated steroids at the concentrations used, suggesting an inhibition of 21-hydroxylation. Inhibition by M of 11- and 18-hydroxylations in rat adrenal mitochondria appears to be linked to the utilization of Krebs-cycle acids and may act mainly on the cytochrome P-450 system rather than the classical electron transport system[45].

Prolonged treatment of dogs with M results in elevated blood pressure, hypokalemia, polyuria and 17-KS excretion in some animals which may have resulted from increased S and DOC secretion[46]. Adrenal hyperplasia in half of the dogs resembles that seen in the hypertensive form of congenital adrenal hyperplasia. Chronic treatment of rats with M and amphenone B causes decreased weight gain and plasma B levels but increased adrenal weights[47]. Peculiarly, injecting only 0.001 μg of B increases ACTH activity of plasma. With increasing amounts of B, the ACTH content rises further and then decreases even if median eminance lesions were made 2 days earlier. Thus, small amounts of B may stimulate the anterior pituitary directly.

SKF-12185. 2-(p-aminophenyl)-2-phenylethylamine inhibits B production *in vitro* by the rat and F production *in vitro* and *in vivo* by the guinea pig adrenal[48]. Signs of adrenal insufficiency are produced in rats. Clinical studies suggest that like M, the drug is partially an 11- and 18-hydroxylase inhibitor[49, 50].

Glutarimide Derivatives. Glutethimide (α-ethyl-α-phenyl glutarimide) as well as its o-, m- and p-$OCH_3$ and p-OH phenyl derivatives are inactive[51]. Dehydrogenation of the ring, reduction of one or both keto-groups or substitution of S for O yields no active compounds. The N-amino, o-amino-

phenyl, and p-amino phenyl (aminoglutethimide) derivatives as well as 3-biphenyl-6-keto piperidine and 3-biphenyl piperidine are found to be total blockers. An 11-hydroxylase inhibitor, α-(p-chlorophenyl)-α-(2-pyridyl) glutarimide is also reported.

Aminoglutethimide (AG) blocked the ACTH response, caused adrenal hypertrophy in rats and normalized plasma and urine corticosteroid levels in 2 patients with Cushing's syndrome[52]. Its use as an anticonvulsant drug in 2 children produced signs of adrenal insufficiency[53]. The secretion of F and B in puppies fell with no rise in S or DOC and it was concluded that AG acts between Chol and Preg[54]. Adrenal, thyroid and ovarian weights increased in normal but not hypoxed rats given AG[55]. During 182 days of treatment all clinical and biochemical signs of Cushing's syndrome disappeared and adrenal metastasis appeared arrested[56].

Steroids. A large number of steroids including adrenal corticosteroids, androgens, estrogens and synthetics were found to inhibit a number of hydroxylations carried out by bovine adrenal homogenates[57]. However, the use of amounts of steroids exceeding their solubilities introduces an element of doubt as to the validity of the interpretations since some of the effects may result from an interaction between a solid phase and enzymes[58].

Norethynodrel, 17-α-ethinyl-17β-hydroxy-5(10)-estrene-3-one, increases adrenal weights in intact female rats[59]. Plasma and adrenal B levels decrease at the time of day when values are normally highest. Clomiphene increases output of 17-oxysteroids in a patient with galactorrhea[60]. Cyanotrimethylandrostenolone, 2α-cyano-4,4,17α-trimethyl-17β-OH-5-androstene-3-one, causes adrenal hypertrophy, a fall in adrenal venous B of male rats and inhibition of 3β-hydroxysteroid dehydrogenase[61]. Norethandrolone, 17α-ethyl-17β-OH-4-norandrostene-3-one, stimulates the production of B by adrenals of castrated rats and increases pituitary ACTH content[62]. This androgen partially reverses the E induced suppression of B production but not of the adrenal response to stress[62]. Testosterone, androstenedione, DHEA, estrone and estradiol has no effect on conversion of B to 18-OH B and Aldo by sheep adrenal mitochondria[8].

Miscellaneous. Other total blockers of adrenal hydroxylations include 1,2- and 1,4-naphthoquinones[51]. Inhibitors of 17-, 18- and 19-hydroxylases include 1- and 4-(5) but not 2-benzylimidazoles[51]. Inactive compounds include imidazolines, benzimidazolines, diphenylhydantoin and various barbiturates[51].

Adrenal necrosis caused by 2-dimethylbenzanthracene (DMBA) is blocked by M or impaired liver function[63] while that brought on by its metabolite, 7-hydroxymethyl methylbenzanthracene is blocked only by M[64]. This metabolite is 2-3 times more active, less toxic and spares the zona glomerulosa (z.g.). Unlike the DMBA, RO-1-8307, N-formyl-chitosan polysulfinic acid, having heparin-like activity, reduces the width of z.g. but not the other zones of the rat adrenal[65]. It reduces Aldo secretion in cases of primary aldosteronism and blocks 18-hydroxylation[66]. A heparin antagonist, hexadimethrine bromide, causes adrenal and pituitary necrosis in the rat but not the dog despite a fall in adrenal blood flow and 17-OH steroid secretion[67].

Stimulation of adrenal function by N,N-dimethyl-p-(m-tolylazo) aniline appears necessary for the production of liver toxicity[68].

Methylcholanthrene increases plasma half-life of B without affecting its volume of distribution suggesting a decreased rate of metabolism[69]. However, when the pool size is calculated it can be seen that the turnover of B is only slightly reduced, if at all. Phenylbutazone increases the volume of distribution with no change in half-life but similar calculations show that the turnover of B is the same as in cold exposure. It is suggested that the increased turnover in cold stress results only from increased synthesis. However, the use of steady state kinetics requires that synthesis and degradation be equal and thus both would increase

Cortisol secretion by isolated adrenal pouches in hypoxed dogs was stimulated by cyclic AMP, vasopressin and ACTH[70]. However, epinephrine and norepinephrine, which stimulate formation of cyclic AMP in fat pads, dichloroisoproterenol, which inhibits adenyl cyclase, or dihydroergotamine, an inhibitor of action of cyclic AMP in the liver, failed to have any effect.

The -SH binding agents, diethyldithiocarbamate, p-chloromercuribenzoic acid, iodoacetic acid, formamidine acetate and N-ethyl-maleimide inhibit 18-hydroxylation of B[8]. Of these, only formamidine acetate fails to inhibit 11-dehydrogenase. Of the 17-hydroxylase inhibitors tested, Su-9055 inhibits 18-hydroxylase, Su-8000 acts on 18-ol dehydrogenase and Su-10603 is inactive with sheep adrenal homogenates[8]. Since Su-9055 and Su-8000 were tested on different homogenates showing appreciable variation in 18-OH B/Aldo ratios, the conclusion that the two drugs differ in action is open to question.

Stimulation of 11β-hydroxylase activity by Krebs-cycle acids is inhibited by CN and Amytal[8]. With rat adrenal homogenates, Amytal is not active in presence of isocitrate or Ca and the CN effect is reversed by isocitrate + NADPH[71]. Oligomycin is inactive but Antimycin A, ferricyanide and dinitrophenol act like Amytal. This supports the concept of a link between the classical electron transfer chain and the P-450 containing hydroxylating system.

Rhythm and Stress. An excellent review of Circadian rhythm includes a section dealing with that of adrenal cortical secretions[72]. In the rat, the plasma and adrenal B are higher in the P.M. and F implants in the median eminance, midbrain reticular formation and ventral hippocampus reduced plasma and adrenal B. However, only the median eminance implant reduced the A.M. adrenal B and adrenal weight[73]. It is felt that the median eminance regulates the secretion of the corticotrophin releasing factor (CRF) while the other areas in some way modulate this effect. Humans excrete more 17-OHCS in the A.M. and in men the increment associated with "sustained effective distress" was greatest at this time[74]. The increment seen with women, however, is greatest during the P.M. trough of the diurnal curve.

Pathological emotional stress and adrenocortical activity is reviewed and it is concluded that the psychological variable correlating with increased secretion is loss of "ego defense strength"[75]. Stress increased the CRF activity of rat plasma[76] but this could be an artefact since CRF was tested in rats with implanted pituitary tumors while ACTH was tested

in normals. If the adrenals were larger in the tumor rats the increment in the ACTH response could be mistaken for CRF activity.

Rats with diabetes insipidus (DI) responded to stress with a smaller increase in plasma B and this went uncorrected after several weeks of vasopressin treatment, despite the improvement of DI[77]. Treatment of rats with the histamine depleter, 48/80, blocked the adrenal response to various forms of stress but not to histamine[78]. This suggests some role for histamine in the stress response and in this regard it should be noted that anaphylactic shock in dogs is accompanied by a stimulation of 17-OHCS secretion[79].

A neonatal stress non-responsive period[80] does not exist when plasma B is measured in place of adrenal B[81, 82].

Action of ACTH. ACTH given in 4 injections to rats decreases adrenal lipids and Chol esters but increases phosphatides and arachidonic acid[83]. Bovine adrenal slices use more added Chol* but not P* in the presence of ACTH[84]. The conclusion that a pool of 21-desoxypregnanes is released by ACTH for hydroxylation is questionable in the absence of data on the time course of production of steroids.

Histochemical analysis of enzymes in rat adrenal sections demonstrated that NADPH-dichlorophenolindophenol reductase of the zona fasciculata-reticularis (z.f-r.) is stimulated by ACTH whereas NADPH or NADH quinone reductase, NADPH and NADH oxidases or NADPH-NAD transhydrogenases are not[85].

In tissue culture, cells of the rat adrenal cortex with mitochondria of the type found in the z.g. do not respond to ACTH while those of the z. intermedia show mitochondrial changes to the vesicular types seen in the z.f-r.[86]. The tubular and vesicular forms of the endoplasmic reticulum also increase. A large number of dehydrogenases are shown to be present but ATP-ase as well as alkaline and acid phosphatase are absent. Chromatin condensation along the nuclear membrane suggest that ACTH might effect transformation of z.g. cells to those of the z.f-r. by action at the nuclear or gene level.

Cloned mouse adrenal cortex tumor cells respond to ACTH with an increased output of steroids which is independent of the growth phase[87]. Within 5 minutes the cells retract from the surface of the vessel and from each other and within 1 hour a rounded-up morphology is seen. This cell line is unable to hydroxylate position 21 but has a high ability to reduce the 20-keto group.

A synthetic $\beta^{1-24}$ corticotrophin stimulates B production by rat adrenal sections[88] and has a half-life in man of 32 minutes[89]. Little or no activity is seen with the $\beta^{1-10}$ and $\beta^{11-24}$ fragments[88]. Alpha-melanocyte hormone has only weak ACTH-like activity[88, 90]. A synthetic $\beta^{1-25}$ corticotrophin with D-serine, L-norleucine and L-valine-amide at positions 1, 4 and 25 respectively was prepared[91] and reported to be 6 times as active as the natural and synthetic $\beta^{1-24}$ preparations[92].

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Chapter 26. 5-Hydroxytryptamine and the Central Nervous System
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Much of the extensive work that has been done on 5-hydroxytryptamine (HT; serotonin) since its isolation and identification twenty years ago has been directed to uncovering its possible function in neural activity. As no short review can include references to all the numerous contributions, the authors have tried to select those references that are recent and include a significant bibliography. No attempt has been made to designate priorities for the observations. Two books[1,2] and a symposium[3] scheduled for publication in 1968 provide full references and detailed discussions of all aspects of the subject.

Distribution in the central nervous system (CNS) - HT, which was first detected in mammalian brain by Twarog and Page[4] and Amin et al.[5] occurs in the CNS of many vertebrate, more primitive species having a relatively greater concentration. The amine is concentrated in the phylogenetically old parts of the brain[5,6] which are connected with the autonomic nervous system or with the reticular activating formation. Thus, the largest amounts of HT are present in the hypothalamus and midbrain, while much smaller amounts are found in the cerebral cortex, cerebellum and white matter[6]. After intraventricular injection, tritiated HT was taken up by areas rich in endogenous HT[7,8]. The use of histofluorescence microscopy in combination with drugs allowed the visualization of central serotonergic neurons in their entire extension[9]. Axotomy combined with histofluorescence microscopy has shown that HT rapidly accumulates in the portion of the axons immediately proximal to the lesion, an observation suggesting that HT is synthesized in the cell bodies and transported down the axons[9-11].

Maintenance of HT (and norepinephrine also) in the rat brain is dependent upon the integrity of the medial forebrain bundle within the lateral hypothalamus[12,13]: When the medial forebrain bundle degenerates as a consequence of a lesion produced in the lateral hypothalamus, HT is depleted in the telencephalon ipsilateral to the lesion in the rat and cat. Concomitant with depletion of HT in this region is a decrease in the activity of L-aromatic amino acid decarboxylase, the enzyme that forms HT from its precursor[14]. Specific lesions can selectively affect monoamine levels: lesions in the central gray area and the septum lower the level of HT in the telencephalon without affecting the level of

norepinephrine, whereas an opposite effect follows ventrolateral tegmental lesions[12]. Such studies have mapped out the central serotonergic system. The ascending serotonergic neurons have their cell bodies located mainly in the mesencephalic nuclei and their axons run uncrossed, principally in the medial forebrain bundle to the limbic forebrain structures and the hypothalamus[15]. The presence of descending HT neurons has also been described in the mammalian spinal cord; the cell bodies are located in supraspinal centers and the fibers descend in the lateral and anterior funiculi to end in the gray matter[9,16].

Differential and density gradient centrifugation of mammalian brain showed that most of the HT is associated with particulate material, especially a fraction containing pinched-off nerve endings[17-21]. Disruption of the nerve ending particles by ultrasound[22] or by osmotic shock yielded a fraction that contains vesicles like those seen at the synaptic junctions by electron microscopy. HT, differing from norepinephrine, does not seem to be present primarily in the vesicle fraction[20,22,23], perhaps because it leaks out of the vesicles during disruption of the nerve endings[24]. The uptake of HT by particulate fractions of brain[25-30] is dependent upon concentration of the amine[26,27,29,30] and upon temperature[27-29], and was not saturable with a high concentration of HT[27,29,30]. Complexes between HT and other biochemicals have been reviewed[21].

Biosynthesis - HT is synthesized in brain from the dietary amino acid L-tryptophan (TP), with 5-hydroxytryptophan (HTP) as an intermediate. TP is actively and stereospecifically transported into brain[31]. Increasing the intake of TP can increase the brain levels of HT[32-35]. Phenylalanine and leucine decrease these levels[36,37] perhaps by inhibiting transport of TP into the synaptosomes (Lovenberg[3]). Formation of HTP from TP, which is the rate-limiting step in the formation of HT, is catalyzed by a hydroxylase specific for TP and requiring NADPH, a reduced pteridine, 2-mercaptoethanol and probably ferrous ion[35,38-41]. The hydroxylation by the pineal enzyme is inhibited by norepinephrine, α-methyldopa, 3,4-dihydroxy-n-propylacetamide, and L-phenylalanine (Lovenberg, et al.[42]). The inhibition of the hydroxylase and the reduction of HT levels by α-n-propylacetamide are not specific[43,44]. A drug that appears to inhibit specifically tryptophan hydroxylase and to deplete brain HT in most species without affecting the levels of catecholamines is p-chlorophenylalanine[45,41,42,3]. But in man the effects of this drug are not clearly attributable solely to depletion of HT[46].

The decarboxylation of HTP to form HT is carried out by a pyridoxal-dependent, non-specific L-amino acid decarboxylase[47-52]

the distribution of which roughly parallels the content of HT in brain[53, 6]. Loss of enzyme activity after lateral hypothalamic lesions occurs in the same areas of brain that show a decrease in HT after these lesions, probably as a result of degeneration of amine-producing neurons[14]. The decarboxylase is found in the soluble portion of the cell[6, 24]. Inhibitors of this non-specific enzyme have been described, the most extensively studied being α-methyldihydroxyphenylalanine[54].

Disposition - It is possible that one mechanism for the disposition of the endogenously produced amine is by diffusion, which could be followed by re-uptake by nerve endings (or by other material in tissues) as has been shown with norepinephrine. But as yet there is no evidence for such a cycle for HT, although, as noted above, brain and its particulate fractions have the capacity for uptake. A main catabolic pathway for HT is oxidative deamination by monoamine oxidase to form 5-hydroxyindoleacetaldehyde, most of which is converted to 5-hydroxyindoleacetic acid (HIAA) by aldehyde dehydrogenase and NAD[55, 56]; a relatively small portion is reduced to 5-hydroxytryptophol by alcohol dehydrogenase and NADH[57, 58]. Monoamine oxidase, which, like aldehyde dehydrogenase[56], is associated with the mitochondria[59], is found throughout the brain, with highest activity in the hypothalamus[6]. Lesions of the medial forebrain bundle, which produce a localized fall in brain HT and HTP-decarboxylase activity[14], also cause a fall in HIAA in the same areas[60]. Similarly, section of the thoracic spinal cord produces a fall in HT and HIAA caudal to the lesion with complete disappearance of both the amine and the metabolite[61]. It is possible that some of the HIAA in brain derives from transamination of HTP, followed by oxidation of the 5-hydroxyindole pyruvic acid, but almost certainly most of the HIAA comes from HT. Other catabolic pathways for HT exist in brain[2], including acetylation of the primary amino group. In the pineal body, the acetylation of the amino group is followed by methylation of the phenolic hydroxyl group to form melatonin[62]; the methylating enzyme has not been found in any other mammalian tissue examined. Also uniquely present in pineal body are 5-methoxytryptophol [63] and 5-methoxyindole acetic acid[64]. The hydroxyindole-O-methyltransferase requires S-adenosylmethionine[62]. Among other postulated catabolites of HT are hallucinogenic harmalan-like compounds (resulting from cyclization of the side-chain) and bufotenine[65]. The presence of the latter in urine has been disputed[66, 67], and its presence in brain not demonstrated.

Numerous inhibitors of monoamine oxidase have been studied, many in man[2]. Their effects cannot be unambiguously attributed to an elevation of HT, because they concomitantly elevate catecholamines. Ingestion of ethanol diminishes the excretion of HIAA and elevates that

of 5-hydroxytryptophol (and its conjugates), presumably due to the effect of ethanol in increasing NADH relative to NAD[68,69].

The turnover rate of HT in brain, estimated by measuring the time required to increase the concentration of HT in brain by 50 per cent, is about 10 to 20 minutes[70]. Measurement of the amount of HIAA formed from HT yielded a half-life of nearly 40 minutes, about 0.4 μg/g/hr[71].

Much of the HT spontaneously released from neural structures can be recovered unchanged from the incubation media containing brain slices[72] or isolated spinal cord[73] or in perfusates of the cerebral ventricles in which the HT is assumed to come from hypothalamus and caudate nucleus[74]. Electrical stimulation of a spinal cord preparation[73] and the medulla oblongata[75] is accompanied by a release of HT. Of special interest is the observation that electrical stimulation of the midbrain raphe, an area consisting essentially of HT-containing neuronal perikarya whose terminals project in various forebrain regions, causes a fall in HT and an elevation in HIAA in the forebrain. Unilateral lesions of the medial forebrain bundle prevent the stimulation-induced changes in indole concentrations only in the forebrain ipsilateral to the lesion, an observation suggesting that the release of endogenous HT is mediated by a specific neural pathway[76].

Numerous drugs have been shown to deplete brain of HT, as well as other biogenic amines[2,21,77]. Reserpine, the prototype of such drugs, may owe its sedative action to the rate at which it depletes the brain of HT[78]. A new agent, p-chloroamphetamine, depletes brain of HT but not of catecholamines[2,3,79,80].

Development and growth - Brain HT is lower in fetal and newborn rats than in the adult and increases in extrauterine life, as do the catecholamines[81]. At birth monoamine oxidase activity is low but the decarboxylase is as active as in the mature rat[82]. The low levels of HT probably result from low activity of the enzyme that hydroxylates TP[82,83]. Also in rabbits[84] and chicks[83], brain HT increases during fetal and early postnatal life, but results on guinea pigs conflict[85,86].

Pineal body and circadian rhythms - The pineal body is rich in biogenic amines, and the formation and metabolism of HT, melatonin and other indoles was noted above[87,88,41]. HT is found there in the pinealocytes and in the sympathetic nerves[89], as contrasted with norepinephrine which is found only in the latter[90] and histamine which is found in mast cells within the pineal body[91]. At least some of the HT found in the sympathetic nerves may be formed by pinealocytes from

which the amine is elaborated and then take up by the nerve endings[89, 3].

Many important physiological actions are now being ascribed to melatonin[89, 42, 3] which is found only in the pineal body. Among these is an influence on pituitary mechanisms controlling secretion of the melanocyte stimulating hormone and luteinizing hormone. Melatonin inhibits the estrous cycle in rats. The estrous cycle is markedly influenced by light, which also decreases the activity of hydroxyindole-O-methyl transferase, the enzyme that forms melatonin. Light increases the activity of HTP-decarboxylase[88].

The diurnal rhythm in the activity of hydroxyindole-O-methyl transferase depends on changes in light, whereas serotonin circadian rhythm is endogenous as it is not abolished by continuous darkness or by blinding, but can be suppressed by continuous light exposure or by superior cervical ganglionectomy or by section of preganglionic fibers to the superior cervical ganglion[88]. Both biological clocks appear to be modulated by nerve impulses generated by photoreceptors (the retina in adult rats), carried through the midbrain and brain stem to the spinal cord, to reach the pineal through a pathway including preganglionic sympathetic fibers to the superior cervical ganglion[92]. One central pathway is the medial forebrain bundle, lesions of which suppress pineal HT (and norepinephrine) rhythms as well as light induced variations of hydroxyindole-O-methyl transferase (Moore and Heller[42, 92]). The pineal bodies of the monkey, kangaroo and pigeon show circadian behavior similar to that of the rat[93]. A circadian rhythm of pinealocyte mitotic activity has also been found in rat[3].

It should be noted that circadian rhythms in HT levels have also been found in the whole brain of the mouse[94] and rat[95], in the cortex of the rat[96], and in the cerebrum and brain stem of the turtle[97].

Effects on CNS - A large number of studies have been conducted on the effects of HT on spontaneous and evoked electrical activity of the CNS[2]. HT, injected into the femoral vein of unanesthetized cats and rabbits, lowers the level of functional activity of the cerebral cortex and subcortical structures, effects that are even more evident after the injection of 5-methoxytryptamine[98]. When HT was injected into the carotid artery of the monkey, cortical synaptic inhibitory actions were seen, which were also seen with LSD-25 and which could be blocked by chlorpromazine; this response of the monkey is qualitatively identical in dogs and cats[99]. In the spinal cord of the cat, HT levels can be markedly increased by an intravenous injection of TP or HTP, with a concurrently large increase of monosynaptic spike height, accompanied by spontaneous motoneuronal discharge, while polysynaptic spiking is

depressed[100]. Similar effects were seen after intracisternal injection of HT[101].

Iontophoretic application[102] of HT prompted inhibitory responses in the neurons in both neo-[103] and paleo-cortex[104] of the cat and on the hippocampal neurons of the cat[105,106] and of the rabbit[107]. Studies on the lateral geniculate nucleus of the cat with this technique have shown that HT depresses the orthodromic excitation of the single geniculate neurons to volleys in the optic nerve fibers, but do not affect antidromic excitation elicited by electrical or chemical stimulation of the optic radiation[108]. Most neurons of the same nucleus are depressed by HT and excited by acetylcholine and norepinephrine, whereas a few cells, very likely of the short-axon type, are excited by HT[109]. The olfactory bulb neurons of the rabbit respond to HT with a slowing of the spontaneous discharge rate[110]. In the hypothalamus of the cat, those cells that were sensitive to HT (and norepinephrine) responded to both amines with a decrease in the rate of firing[111]. Iontophoretic application of HT on the lower lumbar and lumbosacral spinal interneurons have shown that HT has a depressant action[112], although some neurons respond with either facilitation or depression of firing[113].

HT has been implicated in the control of body temperature. The suggestion that normal body temperature is a reflection of a balance between the release of HT and catecholamines in the hypothalamus[114] is complicated by species differences in responses to both HT and catecholamines. Injections of HT into the cerebral ventricles[114] or in the anterior hypothalamus[115] of the cat and dog[116] produce a long-lasting increase of body temperature, accompanied by shivering. HT applied intraventricularly in the goat[117], sheep[118], rabbit[119] and ox[120] produces a fall in body temperature. In contrast with these findings in the rabbit after HT is injected into the ventricle or anterior hypothalamus, injection into the cisterna produces a hyperthermic response[121]. In the rat, p-chlorophenylalanine depleted brain of HT without affecting body temperature[3]. A provocative observation is that a substance released from the hypothalamus of a hypothermic monkey raised the temperature of another monkey when introduced into its third ventricle; the substance released from the hypothalamus, when assayed, was antagonized by bromo-LSD, an antagonist of HT[3].

A role of HT has been postulated[122] in Hess' trophotropic system which integrates the parasympathetic system with activities leading to sleep. Perhaps pertinent to this alleged role is the observed rhythm in HT levels in the brain of mice and rats, the highest amounts being present when rodents sleep[94,95]. Raising brain levels of HT in young chicks by the injection of HT or HTP[123,124] and in cats by drugs[125]

produced electroencephalographic patterns of sleep. The intracarotid injection of HT had the same effect probably by stimulating the bulbopontine hypnogenic areas, via the area postrema[126]. The injection of HTP into one portion of the bulbopontine hypnogenic area of the rabbit produces a slow electrical pattern of sleep, and into another portion of this area a rapid pattern[127].

A most striking correlation between HT telencephalic levels and sleep was presented by Jouvet[3]. When a cat was given p-chlorophenylalanine, a marked depletion of brain HT and total insomnia followed, which could be reversed by the administration of HTP (which by-passes the inhibited step in HT biosynthesis). Destruction of the midbrain raphe, where the majority of serotonergic perikarya are[76], produced a fall in HT in the telencephalon without altering levels of catecholamines. Total destruction of the midbrain raphe produced an arousal pattern with a severe reduction in daily sleeping time from the normal of 60 per cent to 3.5 per cent. The extent of destruction of the raphe nuclei is well-correlated with telencephalic levels of HT, the electroencephalographic pattern, and amount of sleep.

In man, oral administration of TP reduces the duration of the first phase of slow-wave (non-dreaming) sleep and provokes an abnormally early onset of the second phase of paradoxical (dreaming, rapid-eye-movement) sleep. In patients with narcolepsy, where paradoxical sleep is the first phase, this phase is prolonged. In both cases TP effects are blocked by methysergide[128]. Methysergide, an antagonist of HT, blocks the early onset of dreaming sleep[128]; it also causes insomnia[126].

The possibility that HT may be catabolized to hallucinogenic substances was mentioned above. This possibility, together with the observed hallucinogenic activity of many indoles, has prompted the idea that metabolism of HT may play a role in schizophrenia, a subject that has been reviewed[67,129]. One of the provocative observations, first made by Kety and his associates, is that feeding a combination of tryptophan and methionine can induce symptoms of schizophrenia. Studies of the effect of HT on affective states and behavior[1,2,67,129-132] cannot be discussed fairly in a short review. A role of HT metabolism in the mental retardation of phenylketonuria[133] remains unproved, although it has been noted that phenylalanine inhibits transport and hydroxylation of TP[3].

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# Chapter 27. Regulation of Cell Metabolism: Role of Cyclic AMP

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Cyclic 3',5'-adenylic acid (cyclic AMP), the structure of which is shown in Figure 1, was isolated from animal tissue by Sutherland and Rall[1,2], while investigating the mechanism by which epinephrine exerts its glycogenolytic effect in liver and muscle. These and subsequent investigations in various laboratories suggested a role for cyclic AMP as a modifier or mediator of phosphorylase[3,6], UDPG-α-transglucosylase[7,8], phosphofructokinase[9], lipase[10,11,11a], tryptophan pyrrolase[12], steroidogenesis[13-15,15a,15b], ketogenesis[16], amino acid uptake into liver proteins[17], acetate incorporation into liver fatty acids and cholesterol[16], lactate conversion to glucose[18], release of amylase[19], water and ion permeability in the toad bladder[20,21], sugar transport in thyroid tissue[22] and acid secretion in the gastric mucosa[23]. In addition, this nucleotide has been implicated in the actions of glucagon[24], ACTH[3], vasopressin[25], luteinizing hormone[26], thyroid stimulating hormone[27], serotonin[28], acetyl choline[29], prostaglandin[11a], histamine[30], melanocyte-stimulating hormone[30a] and insulin[30b,30c]. Several recent papers have appeared in which the role of cyclic AMP as a hormone mediator or "second messenger" has been described in some detail[31-38] and a suggestion that adenyl cyclase may actually be the adrenergic receptor site has also been made[38a]. It is the purpose of this communication to center discussion on the possible value of cyclic AMP, and test systems for measuring its effects, to the medicinal chemist.

## Synthesis and Degradation of Cyclic AMP

Adenyl cyclase - The synthesis of cyclic AMP from ATP is catalyzed by the enzyme adenyl cyclase, which itself is responsive to hormone stimulation in various tissues[39]. Catecholamines have been shown to stimulate the formation of cyclic AMP in a variety of tissues[31] (Table 1[33]), and adenyl cyclase has been shown to be wide-spread in various tissues[39]. Since procedures for the isolation and assay of this enzyme and measurement of the products of its reaction are available[39,40], direct effects of analogues of ATP and/or cyclic AMP (or hormones) on the enzyme can be determined in an *in vitro* system.

The degradation of cyclic AMP is catalyzed by a specific phosphodiesterase which converts it to adenylic acid[31,41] (Figure 1). Again, the effect of nucleotide analogues or other potential inhibitors on the degradation of cyclic AMP can be studied *in vitro* in this enzyme system, and the possible significance of such agents will be discussed below.

## Cyclic 3',5'-AMP as a Hormone Mediator

The phosphorylase system - While investigating the site of the glycogenolytic action of epinephrine, Sutherland and Cori[42] demonstrated the stimulation of phosphorylase activity by this catecholamine and subsequent studies[2,32,43]

implicated cyclic AMP in this process. Krebs, et al.[32], have recently reviewed the subject of activation of skeletal muscle phosphorylase under the influence of epinephrine, mediated by cyclic AMP. Figure 2, adapted from their paper, summarizes the steps which have been shown to be involved in this process. The direct activation of phosphorylase b kinase in muscle extract can be demonstrated upon the addition of cyclic AMP in vitro[43,44]. The activation of phosphorylase by epinephrine has also been demonstrated in the perfused heart and intact heart in situ[35]. Thus, three enzymatic systems are available which can be studied in isolated cell preparations for effects of analogues or chemically unrelated compounds on either the synthesis or degradation of cyclic AMP or enzymatic activation by this nucleotide.

The phosphodiesterase[41] which cleaves cyclic AMP is inhibited by methyl xanthines and it is entirely possible that some or all of the biological activity observed for these inhibitors of cyclic AMP degradation may be due to this action. For example, theophylline has been shown to increase phosphorylase a activity in the isolated heart and potentiate the effects of epinephrine in this assay[35].

Effects on lipolysis - In a recent article, Butcher[37] reviews the history of their experiments on the role of the cyclic AMP in the lipolytic process. Their investigations showed an increase in the concentration of cyclic AMP upon the addition of epinephrine to fat pads in vitro, followed by a stimulation of the release of free fatty acids. Caffeine, a known inhibitor of the phosphodiesterase which degrades cyclic AMP, acted synergistically with epinephrine on the accumulation of cyclic AMP and the subsequent release of free fatty acids[37,41]. It should be noted that the lipolytic effect of epinephrine was decreased by dichloroisoproterenol, a known inhibitor of the stimulation of adenyl cyclase by catecholamine[29,37]. In spite of this evidence for the role of cyclic AMP as a mediator in the lipolytic process, the direct addition of this nucleotide to fat pads in vitro did not stimulate fatty acid release[45], although penetration into tissue by the nucleotide was low. When the $N^6$-2'-O,dibutyryl ester of cyclic 3',5'-AMP[46] was added to fat pads or fat pads were perfused with this compound, stimulation of tissue free fatty acid synthesis and free fatty acid release was noted. The substituted compound was at least ten times as active in this system as was cyclic AMP per se. The dibutyryl derivative may penetrate tissue more effectively than does the unsubstituted nucleotide, but also it is degraded by the cyclic phosphodiesterase more slowly than is the parent compound [37]. This is indeed an exciting example to the medicinal chemist of a relatively simple chemical substitution which results in a marked change in the ability of a compound to penetrate tissue and withstand the degradation that its progenitor undergoes. At the same time, the substance obviously can be converted, albeit slowly, to the active cyclic AMP and act as a "slow feed" of the latter.

Effects on steroidogenesis - Early investigations[47,48] showed that incubation of beef adrenal slices with ACTH resulted in an increase in the cyclic AMP level in this tissue and that added cyclic AMP stimulated corticoid synthesis in rat adrenal slices. More recently, Karaboyas and Koritz[49] studied the

mechanism of stimulation of corticoid synthesis in rat and beef adrenal cortex slices and concluded that cyclic AMP and ACTH affect the same site in the biosynthetic pathway between cholesterol and pregnenolone. They further suggested that cyclic AMP is an obligatory intermediate in the action of ACTH. In another recent paper, Roberts and co-workers[50] concluded that 5'-AMP (as well as cyclic AMP) stimulates steroid hydroxylation in adrenal mitochondria. The mechanism whereby cyclic AMP enhances steroid hydroxylation in this system is unknown. In their hands, hydroxylation of steroid could not be obtained even at high levels of cyclic AMP if the adrenal mitochondrial system or homogenate did not contain reduced NADP, and they suggest as possible explanations for the action of this nucleotide 1) increased stimulation of the transport of steroid (or co-factor) across mitochondrial membranes or 2) direct activation of the steroid hydroxylase system. Creange and Roberts on the other hand, also studying the mechanism of 11-β-hydroxylation, concluded that cyclic AMP selectively stimulated this process in the adrenal cortex by a mechanism which is independent of glycogen phosphorylation, reduced NADP generation and endogenous corticoid precursors[51].

In 1964, Marsh and Savard[52] reported that luteinizing hormone increased the level of phosphorylase activity in bovine corpora lutea, that this response was specific for the hormone and that a high degree of correlation existed between the extent of phosphorylase stimulation and the degree of progesterone stimulation by luteinizing hormone. In their studies, exogenous cyclic AMP did not stimulate luteal phosphorylase activity. More recently, Marsh and collaborators[53] showed that luteinizing hormone stimulated the production of cyclic AMP in bovine corpora lutea incubated *in vitro* and that this increase preceded progesterone synthesis. Again the effect was reported to be specific for luteinizing hormone. These investigators also demonstrated an increase in the concentration of cyclic AMP in a human corpus luteum stimulated *in vitro* by human chorionic gonadotrophin.

*Other effects of cyclic AMP in vitro* - In a recent paper, Appleman and co-workers[54] concluded that many similarities exist between the ATP-cyclic AMP activation of phosphorylase b kinase and the conversion of glycogen synthetase from the independent to the glucose 6-phosphate-dependent form.

Certain interesting effects on ion and water permeability are also thought to be mediated by cyclic AMP. Handler, *et al.*[26], reported that either arginine vasopressin or theophylline significantly increased the concentration of cyclic AMP in the isolated toad bladder and that the two together were synergistic. Cyclic AMP was also reported to have the same effect as vasopressin on the permeability of the bladder to water and sodium ion and, accordingly, was concluded to act as an intracellular mediator of the vasopressin effect. It is interesting that this nucleotide has also been reported to increase diuresis *in vivo*, as described below.

Although thyroid stimulating hormone (TSH) has been reported to increase levels of cyclic AMP in thyroid homogenates and slices[27,55], the nucleotide did not increase $C^{14}O_2$ production from glucose nor the incorporation of $P^{32}$-phosphate into phospholipids *in vitro*, as was observed with TSH *per se*[56,57]. More recently, Pastan[58] reported a significant stimulation of both of these

processes by the dibutyryl derivative of cyclic AMP at concentrations as low as 50 μg/ml but not by cyclic AMP at five times this concentration. TSH, as expected, stimulated both processes at low concentration. Again, as mentioned above under lipolytic studies, the enhanced activity of the dibutyryl substituted nucleotide should be noted.

## Metabolic Effects of Cyclic AMP in Whole Animals

As mentioned earlier, cyclic AMP has been postulated as the cellular intermediate in the anti-diuretic action of vasopressin[20,21,25,59,60] in isolated dog kidney homogenates and isolated toad bladder. Based on these observations, Levine studied the effect of cyclic AMP on diuresis *in vivo* and reported a prompt anti-diuretic effect after injection of the nucleotide in man[61]. Levine and Vogel[62] also determined the effect of the cyclic nucleotide on heart rate, cardiac output, mean blood pressure, total peripheral resistance, stroke volume, pulse pressure, blood glucose and plasma free fatty acids in intact dogs and reported that administration of a single dose produced effects in the whole animal resembling those of catecholamines. They reported no significant changes in cardiac or metabolite functions following administration of the 2'-,3'- or 5'-AMP, ATP or saline, although prior administration of dichloroisoproterenol prevented the cardiovascular (but not the metabolic effects) of cyclic AMP. The latter nucleotide has also been implicated in the mechanism of myocardial contractility[63-65].

## General Considerations

Examination of available data clearly indicates a role for cyclic AMP as a mediator of the catecholamine stimulation of glycogenolysis, by the mechanism depicted in Figure 2. Unlike the attempts to demonstrate the site of action of most protein and steroid hormones[66], the expected increases in each of the enzyme activities presumed to be mediated by cyclic AMP can be demonstrated with direct *in vitro* techniques after treatment of the appropriate tissue with specific hormones. As an example, adenyl cyclase activity and cyclic AMP increase upon treatment with epinephrine and this in turn can be shown, at the enzymatic level, to activate phosphorylase kinase. The latter enzyme converts phosphorylase b to phosphorylase a which is the active form of the enzyme catalyzing the synthesis of glucose-1-phosphate from glucagon. The opportunities for the medicinal chemist to study analogues and derivatives of the simple nucleotides or of compounds related to the catecholamines are unusual in that several enzymatic steps in a known sequence of reactions can be measured quantitatively *in vitro* and effects of stimulators or inhibitors can be directly assessed. The data implicating cyclic AMP in the lipolytic process are also quite convincing, and the concept of cyclic nucleotide mediation of ACTH in steroidogenesis and vasopressin action on water and ion transport across membranes is also sound. *In vivo*, cyclic AMP exerts many of the effects observed with catecholamines and, in various systems, the methylxanthines (e.g., theophylline), which are known to inhibit the phosphodiesterase that cleaves the nucleotide, also potentiate its action. In addition, the cyclic nucleotide has been implicated in various steps in the gluconeogenic pathway, the mechanism of action of luteinizing hormone, glycogen synthetase and the metabolism of

thyroid tissue.

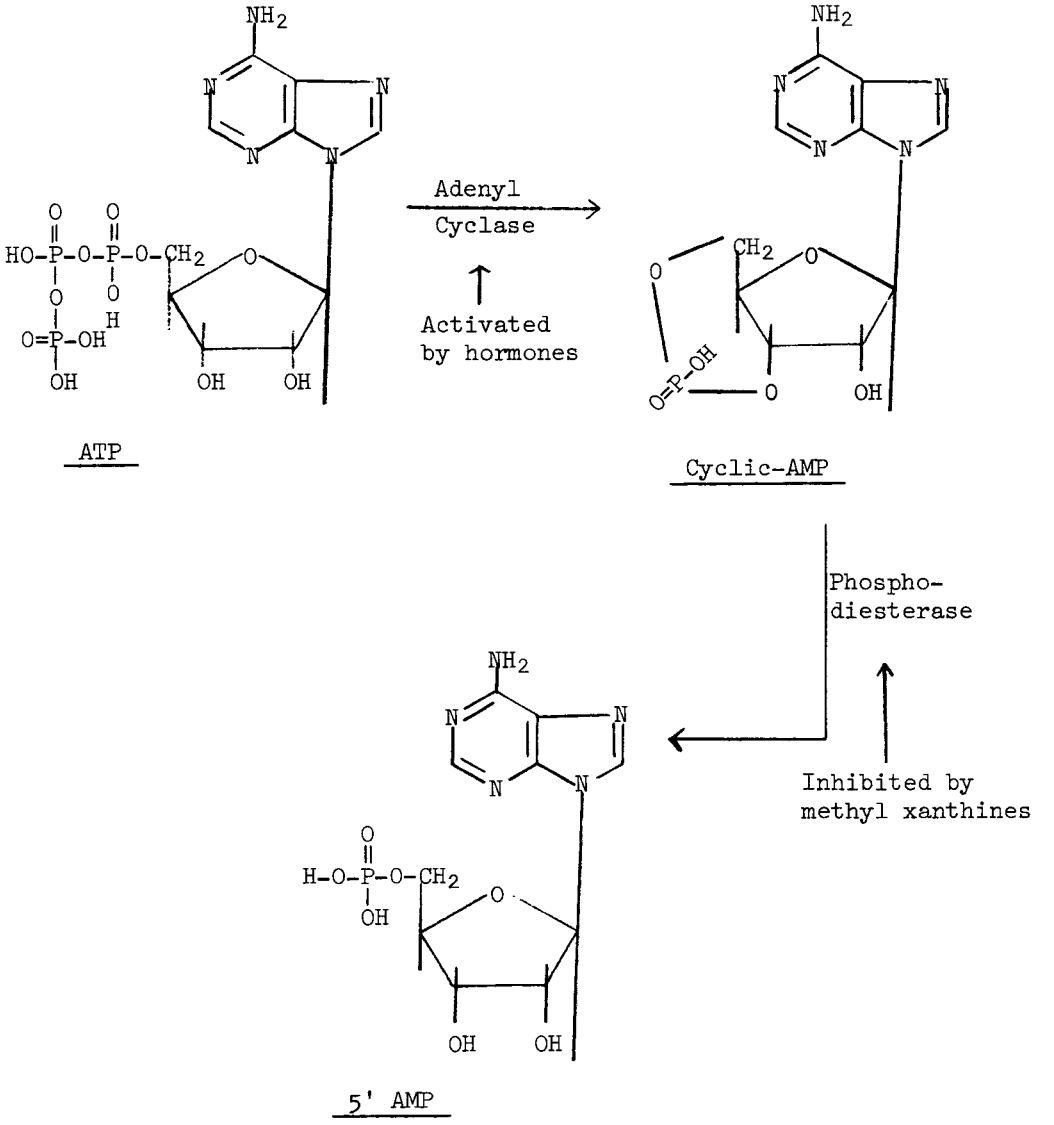
Although it is not uncommon to associate single co-enzymes with different enzymes catalyzing similar types of reactions (e.g., ATP with kinases, NADP with reductases, and the like), it is not common to think of a single small molecule as a mediator of a wide variety of hormonal or metabolic reactions. Since adenyl cyclase is membrane bound, it is possible that stereo-specific attachment sites on the cell surface to which the particular protein or steroid hormone binds activate the synthesis of the cyclic nucleotide in a particular cellular compartment, in which it then affects the activity of another enzyme (e.g., phosphorylase kinase, lipase, or the like), thereby completing the response initiated at the cell surface. Sutherland[67] and Talwar[67] elaborated additional possibilities whereby adenyl cyclase can mediate different hormone effects: 1) different affinities of a single receptor site for different hormones, 2) multiple sites on a single receptor molecule, 3) unique responses in different cells to the activation of adenyl cyclase, 4) different cofactor requirements of the various cyclases, and 5) cellular compartmentalization effects. Regardless of the exact mechanism of action, epinephrine can be shown both in vitro and in vivo to increase the activity of adenyl cyclase and this effect can be stimulated or inhibited by substances which antagonize the synthesis or degradation of cyclic AMP. In the case of lipolysis, there appears to be a direct correlation between the levels of cyclic AMP achieved and the amount of free fatty acid released, which also strongly implicates the cyclic nucleotide as a hormone mediator in this system as well. Coupled with this fact, it must be recalled that cyclic AMP mimics the effect of epinephrine in liver slices and causes hyperglycemia in various intact animals including humans[31].

The finding that N$^6$-2'-O-dibutyryl-3',5'-adenylic acid resists the cyclic phosphodiesterase but penetrates cells more readily than cyclic AMP itself is of great theoretical and practical interest to the medicinal chemist. This represents a situation toward which many investigators strive in preparing substituted derivatives and analogues of pharmacologically active compounds. Very seldom, however, are such clear-cut advantages for a relatively simple derivative realized. It is surprising to this reviewer that considerably more work has not been done to date, both in vitro and in whole animals, with the dibutyryl compound. It is not only imperative that sufficient quantities of this substance be prepared for broad evaluation in experimental animals to determine the overall pharmacological properties of this unique substance, but additional related derivatives with varied side chains also seem worth investigating. It is possible that the preparation of similar substituted nucleosides or nucleotides of bases other than adenine might result in increased permeability in specific tissues and decreased rate of hydrolysis to nucleosides.

Analogues of cyclic AMP per se may also be worthy of investigation in view of the fact that a specific enzyme synthesizes this compound. The enzyme may be localized selectively in various tissues and a specific cyclic phosphodiesterase inactivates the substance by forming 5'-AMP. Thus, enzyme systems in which the medicinal chemist can evaluate compounds in a direct manner for their ability to act in place of cyclic AMP or inhibit its action or degradation

are available. One close relative of cyclic AMP, cyclic tubercidin phosphate[68], has been tested by Butcher and Sutherland (unpublished observations) and shown to replace cyclic AMP as an activator of phosphorylase. Certainly a wide variety of substituted purine analogues and 2'-substituted cyclic AMP derivatives or analogues should be prepared and investigated in the above-mentioned enzyme systems. The ubiquity of cyclic AMP (animal tissues, urine and *E. coli*[69]) and the occurrence of the cyclic monophosphates of guanosine and possibly uridine in nature[70,71] further strengthen the potential importance of these compounds in overall regulation of metabolism, and extend the field of synthesis of possible analogues or derivatives to this entire family of nucleotides. It should be emphasized that selective or specific inhibition of the action of the progenitor substance is not the only basis for interest in derivatives and analogues of compounds related to cyclic AMP. Increased half-life of the molecule in the animal body, allowing it to be carried to specific organs in higher concentration than the parent compound, can be a decided advantage. Likewise, selective organ concentration of derivatives or analogues or changes in rate of cellular kinetics can alter the overall metabolism and distribution picture as well as the biological effects observed with substituted nucleotides[68,72]. Marked changes in polarity of the compound may significantly influence passage across the blood-brain barrier resulting in pharmacologically active doses of a compound in the nervous system where the parent accumulates in only very low concentrations. Obviously, it cannot be predicted whether such changes will be favorable of unfavorable to the animal in which they are induced. When the cyclic AMP and cyclic nucleotide picture is considered *in toto*, it emerges as a fascinating area of biochemistry and biology in which the medicinal chemist ought to play a significant role in tailoring molecules for certain enzymatic activities which can be directly measured *in vitro* and evaluated in various species of whole animals, including man, where indicated.

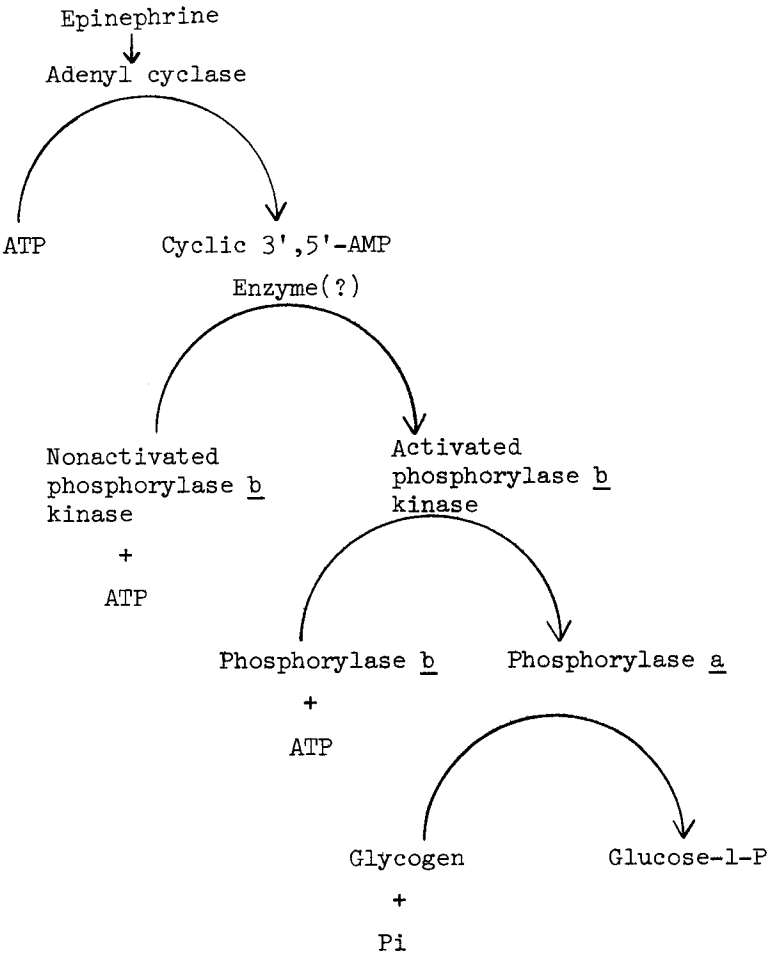
Figure 1




Biosynthesis and Degradation of Cyclic AMP

Figure 2

The Mechanism of Action
of Epinephrine on
Glycogenolysis in Muscle



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Section VI Topics in Chemistry
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## Chapter 28 Synthetic Peptides

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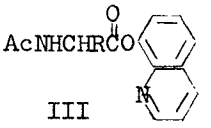
Introduction. This review is based on papers published in 1966, and is necessarily selective in order to keep it short. Some of the newly proposed synthetic procedures are interesting but need further development. Only passing mention will be given to much important work in the field of peptide hormones, since this is covered in the chapter on non-steroidal hormones.

Books and Review Articles. Schröder and Lübke[1] have completed their comprehensive survey with The Peptides, Vol. II. Synthesis, Occurrence, and Action of Biologically Active Polypeptides. This work complements the first volume (Methods of Peptide Synthesis) which appeared in 1965, and the two are recommended as the most comprehensive treatise on peptides available today. A less comprehensive but very readable Peptide Synthesis, by Bodanszky and Ondetti[2] also appeared in 1966, as did a monograph by Kopple,[3] Peptides and Amino Acids, which was written for the use of undergraduate students of chemistry. Not to be overlooked are general reviews by Wieland and Determann[4] and Law[5] on recent work, and more specialized reviews by Russell[6] on Cyclodepsipetides and by S. G. Waley[7] on Naturally Occurring Peptides. A symposium on Hypotensive Peptides, held in 1965, was reported in 1966.[8]

Active Esters. The synthesis of reactive esters of acylamino acids and their use in lengthening a peptide chain by reaction at the amino end is a justifiably popular method; racemization is avoided, yields are good, and purification of the products is relatively easy. Esters of N-hydroxysuccinimide (I), N-hydroxypiperidine (II) and 8-hydroxyquinoline (III) have received particular attention during 1966.


I


II




III

The practical value of N-hydroxysuccinimide (HOSu) esters of acylamino acids is illustrated by their use in the syntheses of insulin components

by the Aachen group.[9-15] Recent improvements of the mixed anhydride method[16] have been adapted to the synthesis of HOSu esters of acylpeptides without racemization,[17] which should greatly extend the utility of these esters. The same communication reported the synthesis of N-hydroxypiperidine (HOPip) esters without racemization; however, the low reactivity of such esters[18] is to their disadvantage. The authors of the latter reference found useful reactivity of HOPip esters of acyldipeptides with amino acid esters, but not with peptide esters. In contrast, 8-hydroxyquinoline (HOQ) esters of acylamino acids and acyldipeptides were found to be highly reactive to amino acid or peptide esters;[19] esters derived from 5-chloro-8-hydroxyquinoline were even more reactive.[20] Since these esters are especially resistant to racemization, they are promising.

Illustrative of the complexity in evaluation of active esters, it has been observed that the mixed anhydride procedure which gives a HOSu ester without racemization yields a partially racemized HOQ ester.[17] There are other indications that HOSu has a good balance of properties for active ester use. Thus, it has been found that the trifluoroacetate esters of p-nitrophenol, 2, 4, 5-trichlorophenol and HOSu are useful reagents for making the corresponding active esters of acylamino acids, but only the HOSu derivative gave little racemization in a sensitive test case.[21] The use of HOSu to prevent racemization by the dicyclohexylcarbodimide method, which likely proceeds via the active esters, will be discussed below. Finally, an ingenious synthesis of t-butyloxycarbonylamino acids via t-butyloxycarbonyl N-hydroxysuccinimide ester and the subsequent in situ synthesis of t-butyloxycarbonylamino acid N-hydroxysuccinimide esters is noted.[22]

An improved procedure for the use of t-butyloxycarbonylamino acid p-nitrophenyl esters in solid phase synthesis of peptides was reported.[23] An opposite procedure, in which the active ester was attached to a resin, was investigated in two laboratories[24,25]; further work will be necessary to evaluate the utility of this approach.

More evidence that commonly used active esters can be used without racemization was found by Weygand, Prox and König.[26] Their test, which involved the coupling of Z·Leu-Phe·OH (2L) with H·Val·Ot·Bu, was applied to a number of common methods; only the azide and the active esters gave no racemate. However, Liberek and Michalik[27] have found that racemization occurs in coupling of Z·ala(CN)·SΦ with H·Gly·OEt; this is an unusual case, but it illustrates that no absolute conclusions can be drawn.

Mixed Anhydride Coupling. This very popular method, involving the use of alkyl chloroformates as reagents to make anhydrides with acylamino acids or acylpeptides, has been reinvestigated. Determann, Heuer, Pfaender and Reinartz[28] have shown that exposure of the mixed anhydride $X\cdot Phe\cdot O\overset{O}{\overset{\|}{C}}OCH_2CH_3$ to triethylamine gives racemization rates in the order benzoyl > acetyl > formyl for X; no racemate was found when X was ethoxycarbonyl or t-butyloxycarbonyl. These results confirm previous findings, and an azlactone mechanism of racemization. Anderson, Zimmerman

and Callahan[16] presented evidence for tertiary amine participation in mixed anhydride formation, with steric factors being important. Racemization could be avoided in sensitive test cases by use of a tertiary amine containing an N-methyl group, provided no excess was used. With a suitably weak amine such as N-methylmorpholine, an excess was permissable. Coupling of acylpeptides without racemization should now be possible, which will considerably extend the utility of the mixed anhydride method.

Dicyclohexylcarbodiimide (DCCD) Coupling. This method was improved by Wünsch and Drees[29] and Weygand, Hoffmann and Wünsch[30]. The first authors found that the addition of an equivalent or more of N-hydroxysuccinimide to the coupling of a C-terminal leucine pentapeptide to a tripeptide ester in the presence of DCCD raised the yield from 31% to 75% and eliminated the N-acylurea byproduct. The latter authors found that racemization in the synthesis of Z·Leu-Phe-Val·OtBu from Z·Leu-Phe·OH and H·Val·OtBu by DCCD was eliminated by using two equivalents of HOSu and a temperature of -22°. Since an excess of DCCD was also used, the minimum requirements for no racemization were not clear. p-Nitrophenol in place of HOSu did not eliminate racemization. In another paper[31], it was found that the coupling of βMZ·Leu-Phe·OH(where βMZ is β-methoxybenzyloxycarbonyl) to H·Val·$OCH_2$·P (where P is a polymer) in dimethylformamide at room temperature in the presence of HOSu (2 equivalents) and DCCD gave some racemization, but a similar reaction in methylene chloride gave none. The use of optically pure βMZ·Leu-Val·OSu also gave no racemate. The implication is that racemization-free DCCD reaction also proceeded through the HoSu ester, but it is not proven.

In work reported at a symposium in 1965 but published in 1966[8] Katchalski reported good results in the synthesis of BOC·Tyr(Bzl)-Ala-Glu(OBzl)·OSu by the DCCD method, removal of protecting groups and polymerization. In retrospect it seems likely that racemization did not occur.

Rapid Synthetic Procedures. The most interesting development of the year was the skillful use of α-amino acid N-carboxyanhydrides in a rapid synthesis in aqueous medium[32]. This procedure is much faster than the Merrifield "solid phase" synthesis, undoubtedly more economical, and probably easier to adapt to larger scale syntheses. It appears that more byproducts are formed, but both methods require chromatographic or more elaborate purification of the final products.

The "solid phase" method was further applied during the year by Merrifield and associates. The most severe test was its use for the synthesis of the A and B chains of insulin[33]; it is not possible to accurately evaluate the results from the brief report, but insulin activity was obtained by oxidative combination of the two chains. In another paper[34] it was shown by synthesis of bradykinylbradykinin, an 18 amino acid peptide, that the o-nitrophenylsulfenyl group can be used for amine protection in this procedure. The automated solid-phase synthesis of the C-terminal decapeptide fragment of tobacco mosaic virus protein was reported by Stewart, Young, Benjamini, Shimiza and Leung[35].

Amine-Protecting Groups. The o-nitrophenylsulfenyl (NPS) group, introduced in 1963[36], received a good deal of attention during the year. Selective removal in the presence of commonly used groups such as benzyloxycarbonyl and t-butyloxycarbonyl (BOC) is a particularly desired property, and the NPS group has been promising for this use. From comparative syntheses of a decapeptide, Poduska[37] concluded that selective removal of the NPS group by hydrogen chloride with BOC as the "stable" group was risky in long syntheses. Kessler and Iselin[38] reached similar conclusions; they found promising results with thioacetamide in the presence of acetic acid, although byproducts from the NPS caused some difficulties. Brandenburg[39] found that acidolysis in the presence of mercaptoethanol helped avoid unwanted byproducts. Fontana et al.[40] recommend thiophenol or thioglycollic acid.

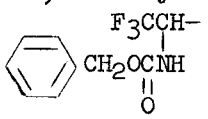
Further work[41] with the t-amyloxycarbonyl group showed that it is comparable to the t-butyloxycarbonyl group. The furfuryloxycarbonyl group was found to be between the t-butyloxycarbonyl and benzyloxycarbonyl groups in ease of removal by acid treatment.[42]

Carboxy-Protecting Groups. Stewart[43] has investigated 2, 4, 6-trimethylbenzyl esters. They are comparable to t-butyl esters in sensitivity to acid catalyzed cleavage. Methanolic hydrogen chloride does not affect them under conditions which remove NPS or trityl groups.

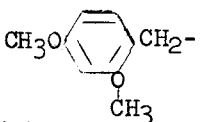
Further work with diphenylmethyl,[44] phenacyl[44], and β-methylthioethyl[45] esters disclosed useful combinations with N-protecting groups, particularly trityl and o-nitrophenylsulfenyl groups.

Special Protecting Groups. In three papers[46,47,48] Hiskey and co-workers reported a study of the problem of SH protection in the synthesis of cysteine peptides. Mercuric acetate was found to be useful for selective cleavage of the S-triphenylmethyl groups, and sodium ethoxide for the S-benzoyl group. The latter group is limited in utility, however, because of tendency to migration to the amino group during peptide bond formation. Guttmann[49] found that a new S-protecting group, ethyl carbamoyl ($CH_3CH_2NHCO$-), was stable under acidic and neutral conditions but readily cleaved by basic reagents; glutathione and oxytocin were synthesized with its use. Wilchek et al.[50] solved the problem by synthesizing peptides containing L-β-chloroalanine and converting these to cysteine peptides with appropriate thio compounds.

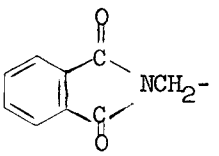
Weygand and associates[51] introduced a new protecting group for the imidazole of histidine, 2, 2, 2-trifluoro-1-benzyloxycarbonylaminoethyl- (IV); it is resistant to acid and to alkali, and is cleaved by hydrogenation, then hydrolysis.




(IV) Z·TF group




(V) DMB group




(VI) phthalimidomethyl

Weygand et al.[52] also proposed the 2, 4-dimethoxybenzyl group (V) for protection of amide groups; perhaps the most interesting aspect of such amide protection is the resulting increase in solubility of peptides in organic solvents. The group is removed by strong acid treatment or by sodium in liquid ammonia.

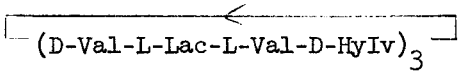
Wilchek et al.[53] found the phthalimidomethyl group (VI) to be useful for protecting the γ-carboxyl of glutamic acid; it was removed by molar piperidine.

Biological Activity of Synthetic Peptides. The primary reasons for the synthesis of peptides are to confirm structures of biologically active natural peptides and to study the effect of modification of such structures on the biological effects. A practical goal is the synthesis of peptides which can be used for drug purposes. Progress in these areas was made in 1966.

Much work on the synthesis of hormones and analogs was reported, and this will be covered in another chapter as already mentioned. A complete report on the syntheses of β-corticotropins by Schwyzer and Sieber[54] is noted here; this is of general interest because of the discussion of problems in synthesis and purification of products.

An analog of a 25 amino acid corticotrophin (ACTH) fragment with improved properties for drug use was reported[55]; resistance to amino-peptidase activity by providing a D-serine residue at the amino end and to carboxypeptidase by a C-terminal valinamide, and resistance to air oxidation by substituting norleucine for methionine were obtained. Biological assays showed increased potency over natural ACTH[56], and more prolonged action in human beings[57]. These results suggest that synthetic variations will be valuable in improving the medical properties of other biologically active peptides.

Replacement of amide by ester linkages gives a class of compounds known as depsipeptides. An example is the cyclic antibiotic valinomycin (VII),




VII

which is composed of valine, lactic acid and α-hydroxyisovaleric acid units. In a paper of special interest[58], Shemyakin and associates showed that replacement of one of the hydroxy acids with a similar amino acid gave compounds with antibiotic activity which in some cases exceeded that of the natural compound. Similarly, replacement of amide by ester groups to give analogs of glutathione, ophthalmic acid and bradykinin gave biologically active compounds. Resistance to enzymatic attack is one of the interesting possibilities of such compounds.

Replacement of amino acid moieties in biologically active peptides by other amino acids with retention of activity is common, and more examples were reported during the year. A particularly interesting study was made

by Hofmann and Bohn.[59] Histidine appears to be important in the biological activity of several enzymes and hormones, probably because of the acid-base behanior of its imidazole fragment. Hofmann and Bohn replaced the histidine of an S-peptide fragment of ribonuclease with β-(pyrazole-1)-L-alanine and with β-(pyrazole-3)-L-alanine; although these are isosteric with histidine, biological activity was lost. Since the pyrazoles differ in acid-base properties, the results suggest that the ionization behavior of histidine is important in the activation of ribonuclease S-protein by S-peptide.

In the antibiotic field, Vogler and Studer[60] have reviewed the chemistry of the polymyxin antibiotics. Studer, Lergier and Vogler[61] reported the synthesis of Circulin A, and Ohno and coworkers[62] synthesized tyrocidin A.

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# Chapter 29. Nucleosides and Nucleotides

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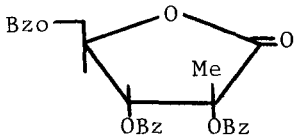
During 1966, a review of methods in nucleoside syntheses[1] and a review of the ionization and metal complex formation of adenosine and adenine nucleotides were published.[2]

The preparation of nucleosides by the fusion method continues to be employed extensively. Iodine has been found to be a good catalyst for the synthesis of certain nucleosides by the fusion method.[3] Nebularine has been synthesized in high yield by the fusion technique using bis-(p-nitrophenyl)hydrogen phosphate as the catalyst.[4] The synthesis of 2'-C-methyladenosine has been accomplished from the protected 2-C-methyl-D-ribono-$\gamma$-lactone (I) which was reduced with bis-(3-methyl-2-butyl)borane-(disiamylborane). The corresponding tetrabenzoyl derivative was converted into the chloro sugar which after condensation with chloromercuri-6-benzamidopurine followed by deblocking gave 2'-C-methyladenosine.[5] This compound as well as 3'-C-methyladenosine are both cytotoxic against KB cells in culture.[5] Nucleosides were prepared by the fusion method from homoribose with 6-chloro-, and 2,6-dichloropurines. The corresponding 6-mercapto- and 2-chloro-6-aminopurine nucleosides were nontoxic in tissue culture (KB and $HE_p$-2/MP).[6] A variety of aldofuranosyl nucleosides have been prepared by the chloromercuri procedure.[7] The utilization of the 2,4-dinitrophenyl group as a blocking group in the preparation of 2'-amino-2'-deoxy-nucleosides has been described in the synthesis of 2'-amino-2'-deoxy-adenosine.[8] Urbas and Whistler have prepared several 1-(4-thio-D-ribofuranosyl)pyrimidine nucleosides by the Hilbert-Johnson procedure.[9] In all instances a 2:1 mixture of anomers was obtained with the $\beta$-D anomer predominating over the $\alpha$-D anomer. The synthesis of 4'-acetamidoadenosine has been accomplished by the condensation of 4-acetamido-1,5-di-O-acetyl-2,3-di-O-benzoyl-4-deoxy-D-ribofuranose with chloromercuri-6-benzamidopurine using a titanium tetrachloride catalyzed reaction.[10]




I

1-Deaza-6-methylthiopurine ribonucleoside and 3-deaza-6-methylthiopurine ribonucleoside have been prepared by fusion of the corresponding deaza-6-chloropurine with tetra-O-acetylribofuranose followed by reaction with sodium methyl mercaptide.[11,12] Both of these deaza nucleosides were much less cytoxic than 6-methylthiopurine ribonucleoside, but 1-deaza-6-methylthiopurine ribonucleoside was cytotoxic to $HE_p$-2 cells which were resistant to 6-mercaptopurine.[11] 3-Deazaadenosine has been synthesized by a related procedure.[13] Replacement of the 6-chloro group could not be accomplished with ammonia. However, treatment of 3-deaza-6-chloropurine ribonucleoside with hydrazine followed by reduction with Raney nickel gave 3-deazaadenosine.[13]

Montgomery and Thomas have studied the infrared, ultraviolet and NMR

spectra of a number of mercury or chloromercury purines and compared these spectra with the spectra of the corresponding sodium salts and N-7 and N-9 alkylpurines.[14] Based on these studies, they concluded that the mercury or chloromercuri are covalent. The chloromercury group is attached to N-7 of 3-benzylhypoxanthine and the mercury group is at N-7 of theophylline. The chloromercuri group is attached to N-9 of 1-benzylhypoxanthine, 1-benzylpurine-6(1 H)-thione, and 6-dimethylaminopurine. When these mercury or chloromercury derivatives are allowed to react with acylglycosyl halides, the attack is on the nitrogen bearing the mercury or chloromercury group.[14]

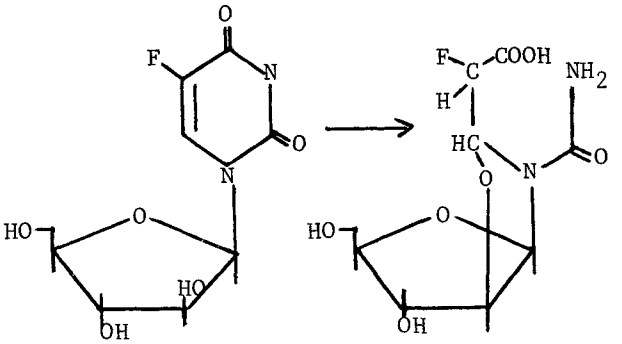
The utility of a 3-substituent on hypoxanthine to direct substitution to the 7-position has been shown by the preparation of 7α- and 7β-D-arabinofuranosylhypoxanthines.[15] Thus, 3-benzyl- or 3-benzhydryladenines were treated with nitrosyl chloride to give the corresponding 3-substituted hypoxanthines. The chloromercury derivatives of these compounds were condensed with 2,3,5-tri-O-benzoyl-D-arabinofuranosyl bromide. Catalytic hydrogenolysis of the benzyl or benzhydryl group gave 7α- and 7β-D-arabinofuranosylhypoxanthines.[15] The synthesis of 7-glycosylpurines as the exclusive product of the reactions is exemplified by the preparation of 7-β-D-ribofuranosyladenine.[16] 4(5)-Bromo-5(4)-nitroimidazole was fused with tetra-O-acetyl-β-D-ribofuranose to give a N-glycosyl derivative which on treatment with potassium cyanide, then Raney nickel and finally with acetic anhydride and triethyl orthoformate gave 7-β-D-ribofuranosyladenine.[16] Shimizu and Miyaki have shown that the migration of the ribosyl group in N-benzoyl-3-β-D-ribofuranosyladenine from N 3 to N 9 occurs by an intermolecular mechanism.[17]

An interesting stereoselective synthesis of the anomeric 5-mercapto-2'-deoxyuridines has recently been reported.[18] When 5-acetylmercapto-2,4-O-bis-(trimethylsilyl)uracil and the blocked chloro sugar were fused at 100-110° for 15-20 minutes, only the β-isomer of the blocked compound could be isolated from the reaction. However, when the reaction was carried out in benzene solution at 37° for 90 hours, only the α-isomer was isolated.[18]

By modifications of known procedures, the following compounds have been prepared: 1-β-D-arabinofuranosylthymine,[19] 2'-deoxy-5-(trifluoromethyl)-uridine and its α-anomer,[20] α- and β-5-trifluoromethyl-6-aza-2'-deoxyuridines,[21,22] 1-β-D-arabinofuranosyl-5-fluorouracil,[23] 5-fluoro-2'deoxycytidine,[24] 3'-deoxynucleosides of pyrimidines,[25] pyrimidine and purine nucleosides of D-glucuronic acid,[26,27] 9-D-mannofuranosyladenine,[28] 3-β-D-arabinofuranosyladenine,[29] 3-β-D-ribofuranosylorotic acid,[30] 5-(D-ribofuranosyl)-6-azauracil,[31] and 5-α-D-arabinitoluracil and 5-α-D-ribitoluracil.[32]

Chemical transformations of nucleosides have resulted in the syntheses of some 2',3'-unsaturated pyrimidine nucleosides[33] as well as a 4',5'-unsaturated pyrimidine nucleoside.[34] Treatment of 3'-O-tosyl-2'-deoxyadenosine with alkoxide produced the 2',3'-unsaturated nucleoside[35,36] as well as a smaller amount of the 3',5'-oxetane derivative.[36] The syntheses of 2',3'-dideoxy-, 2',5'-dideoxy-, and 2',3',5'-trideoxyadenosine have been accomplished by mono- or ditosylation of 2'-deoxyadenosine

followed by displacement of the tosylate with sodium ethyl mercaptide and Raney nickel desulfurization.[37]



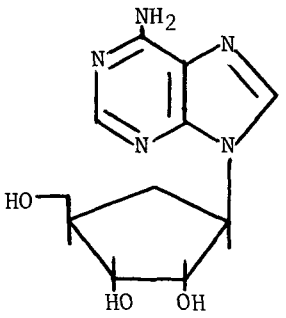
A novel transformation in aqueous alkali has been observed with 1-β-D-arabinofuranosyl-5-fluorouracil as outlined.[38] The open chain compound was isolated in 50% yield and by treatment with diazomethane and then with alkali generated 1-β-D-arabinofuranosyl-3-methyl-5-fluorouracil. Moreover, the ribo- or 2'-deoxyribo derivatives did not undergo this reaction.[38] The acid catalyzed solvolysis of pyrimidine nucleosides has been studied and the acid stability is markedly influenced by the number of hydroxyl groups in the sugar. In the 2'-deoxyribonucleosides, the 3'-hydroxyl group exhibits a large effect on the rate of reaction.[39] A convenient synthesis of 1-β-D-arabinofuranosylcytosine has been reported by tosylation of $N^4,O^{3'},O^{5'}$-tri-O-acetylcytidine. The proposed 2,2'-anhydrocytidine derivative undergoes rapid hydrolysis to 1-β-D-arabinofuranosylcytosine.[40] Hampton and Nichol have described a convenient synthesis of 2,2'-anhydro-1-β-D-arabinofuranosyluracil by treating uridine with diphenyl carbonate.[41]

In a continuation of some studies on acyl migrations in model compounds related to aminoacyl-S-RNA, a technique has been developed using NMR to determine the rate of acyl migration in some 3'-O-acyl ribonucleosides. It was estimated that the half-time of equilibration into 2' and 3'-isomers of an average aminoacyl-S-RNA derivative in pH 7 buffer at 37° is $2 \times 10^{-4}$ sec. It was concluded that equilibration of an average aminoacyl-S-RNA would most likely be much faster than peptide bond synthesis.[42] Two studies of ORD on pyrimidine nucleosides have appeared which assist in the assignment of the anomeric configuration.[43,44] The anomeric configuration can be assigned utilizing an NMR technique which compares the pyrimidine nucleoside with its 5,6-dihydroderivative.[45]

In some 6-substituted purine 3'-deoxyribonucleosides, a direct correlation between the extent of phosphorylation of the nucleosides and inhibition of RNA synthesis in intact Ehrlich ascites cells has been shown.[46] The synthesis and properties of some substituted 6-hydroxylaminopurines has been described.[47]

The structures of formycin,[48] formycin B,[48] and blasticidin S[49] have been described. The syntheses of some nucleosidic peptides related to amicetin have also been reported.[50]

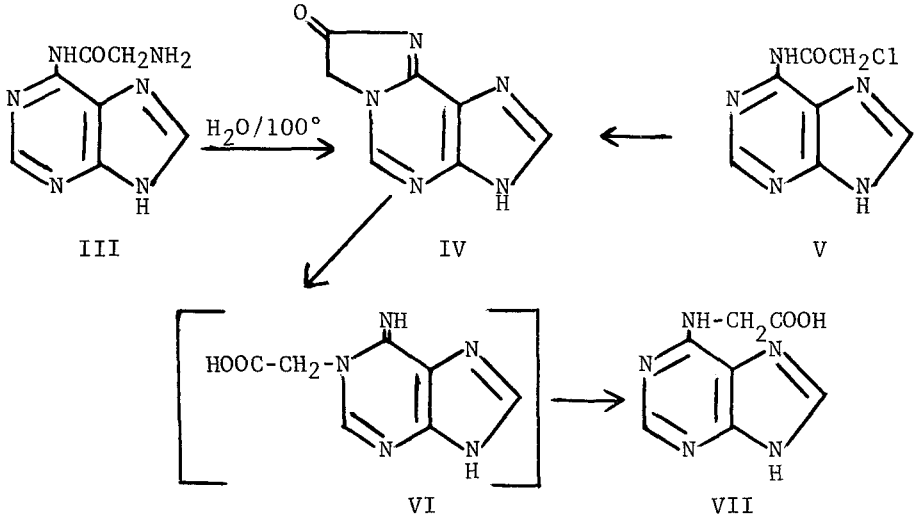
In an elegant paper, Shealy and Clayton have outlined their synthesis of 9-[β-DL-2α, 3α-dihydroxy-4β-(hydroxymethyl)cyclopentyl]adenine (II)



II

which is the carbocyclic analog of adenosine.[51] Norbornadiene was cis-hydroxylated and after protection the olefin was oxidized to yield 2α,3α-diacetoxy-1β,4β-cyclopentanedicarboxylic acid. The cyclic anhydride was converted into the amide acid which on Hofmann rearrangement followed by lithium borohydride reduction on the ester gave a derivative of 1β-amino-2α,3α-dihydroxy-4β-hydroxymethylcyclopentane. Condensation of the aminotriol with 5-amino-4,6-dichloropyrimidine followed by ring closure with triethyl orthoformate gave the 6-chloropurine derivative which on treatment with ammonia gave II.[51]

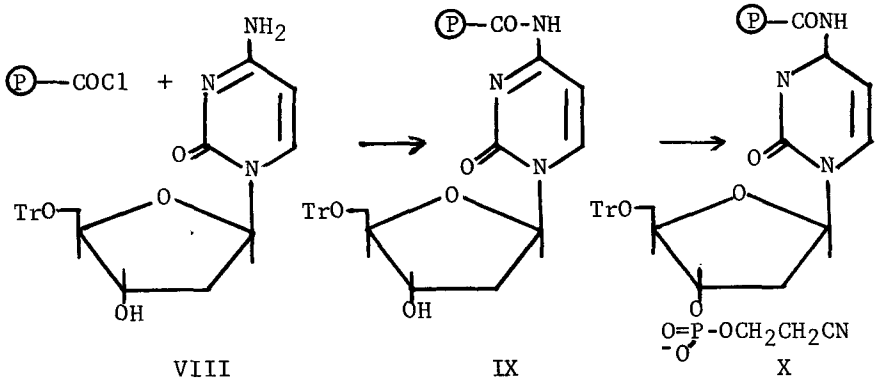
A most unusual rearrangement has been observed by Chheda and Hall with the finding that $N^6$-glycyladenine (III) is converted into N-(6-purinyl) glycine (VII).[52] When III is kept in aqueous solution for a few minutes at 100°, it loses the elements of ammonia and forms a cyclic intermediate which has been assigned structure IV. $N^6$-chloroacetyladenine (V) forms the identical intermediate (IV). Compound IV in neutral solution undergoes ring cleavage, and rearrangement to VII, presumably via VI.[52]



III IV V

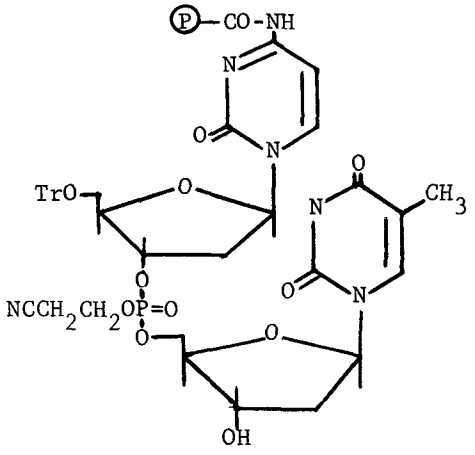
VI VII

The initial announcement of a method for the stepwise synthesis of oligodeoxyribonucleotides on an insoluble polymer support was made in 1965 by Letsinger and Mahadevan.[53] In 1966 several papers appeared on syntheses with a polymer support.[54-56] The procedure used by Letsinger and Mahadevan employs an insoluble copolymer of styrene (88%); p-vinylbenzoic acid (12%) and p-divinylbenzene (0.2% or 0.02 %). The carboxyl groups on the polymer were converted into acid chlorides with thionyl chloride and allowed to react with 5'-O-trityl-2'-deoxycytidine (VIII) to yield IX. Condensation of IX with cyanoethylphosphate and mesitylenesulfonyl chloride or DCC gave X. When X was activated with mesitylenesulfonyl chloride and



VIII IX X

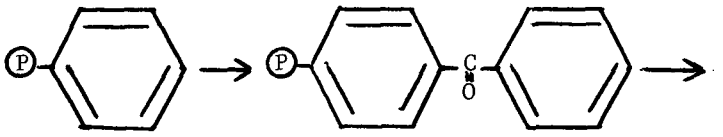
condensed with thymidine XI was produced. By repetition of the phosphorylation of the free 3'-hydroxyl group, activation and condensation steps several different oligodeoxyribonucleotides were synthesized. The final products can be removed from the polymer with 0.4 M sodium hydroxide in a mixture of dioxane, ethanol and water.[53,54]



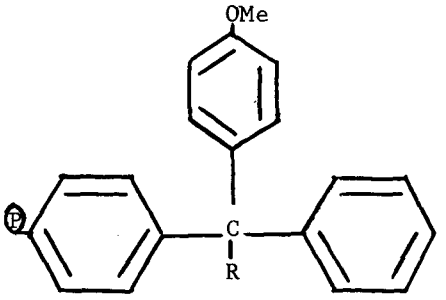
XI

The second method of deoxyribonucleotide synthesis on a polymer support is different from the procedure of Letsinger and Mahadevan in two respects. The polymer employed is a polystrene polymer which is soluble in the reaction medium and the deoxyribonucleoside is attached to the polymer through its 5'-hydroxyl group by means of a methoxytrityl group on the polymer.[55,56] In order to functionalize the polymer (XII), it was subjected to a Friedel-Crafts with benzoyl chloride to give XIII. Treatment of XIII with p-methoxyphenylmagnesium bromide gave the trityl alcohol (XIV) which was converted to the trityl chloride (XV) with acetyl chloride. Condensation of XV with thymidine[55] or 3'-O-acetylthymidine[56] gave XVI. For internucleotide bond synthesis, XVI was allowed to react with pyridinium 3'-O-acetylthymidine 5'-phosphate and mesitylene chloride in pyridine solution. After treatment with water followed by removal of the 3'-O-acetyl blocking group, XVII was obtained in good yield. By repetition of the condensation and deblocking steps, several different oligodeoxyribonucleotides were prepared. Removal of the oligodeoxyribonucleotide from the polymer was accomplished by brief treatment with trifluoroacetic acid in chloroform[55] or dioxane.[56]

A review has appeared on the synthesis and biological function of nucleotides.[57] A paper describing the synthesis of the 64 possible ribotrinucleotides derived from the four major ribomononucleotides has been



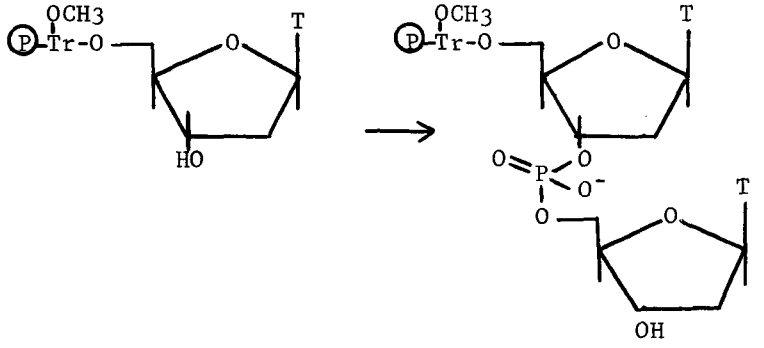
XII XIII



XIV, R=OH
XV, R=Cl

published.[58] A report has appeared describing the use of nucleoside 2'-O-benzyl ethers in the synthesis of oligoribonucleotides.[59] The use of 1-dimethylamino-1,1-dialkoxymethanes as a blocking group for reactive amino groups of cytidine, adenosine and guanosine in the stepwise synthesis of ribooligonucleotides has been described.[60]

Detailed kinetic studies on internucleotide bond synthesis using arylsulfonyl chlorides are reported, and the use of 2,4,6-triisopropylbenzenesulfonyl chloride for internucleotide bond synthesis is described.[61] Phosphorylation of nucleosides with organic amine salts of phosphoric acid has been



XVI XVII

studied.[62,63] Additional studies on the preparation of nucleotides and dinucleoside phosphates by the anhydronucleoside method have been reported.[64,65] Finally, an interesting phosphorylation of ribonucleosides with phosphorus trichloride has been discovered.[66] Reaction of 2',3'-O-isopropylidene inosine with phosphorus trichloride in acetone solution followed by an aqueous treatment and removal of the blocking group gave 5'-IMP in a 91% yield. This reaction was applicable to other nucleoside isopropylidene derivatives. The reaction requires oxygen and a solvent which is a ketone or aldehyde (acetone and methyl ethyl ketone are best). It is postulated that phosphorus trichloride reacts with the nucleoside to give a dichlorophosphite which is then oxidized to the dichlorophosphate.[66]

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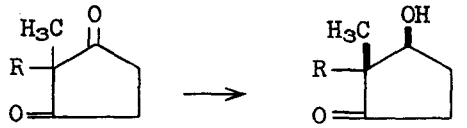
# Chap. 30 STEROIDS

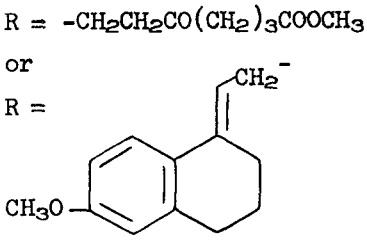
Raphael Pappo, G. D. Searle & Co., Chicago, Illinois

Introduction. Interest in the field of steroids remains undiminished. A major breakthrough was achieved in the elucidation of the biosynthesis of sterols. Strong evidence has been obtained showing that squalene 2,3-oxide is an intermediate in the conversion of squalene to cholesterol.[1,2,3,4]

Other important contributions were in the area of total synthesis. Compounds of type I were converted to the required optically active intermediates II in about 70% yield,[5,6] using microbiological transformations.




(I) d (II)




Thus it has been possible for the first time to effect an asymmetric total synthesis of steroids.

Androstane Series. The selective reduction of steroids by homogeneous catalytic hydrogenation has been studied and shown to be promising.[7,8] A new angular methylation procedure, at $C_{19}$, involving the Simmons-Smith reagent was discovered, thus extending the usefulness of the Smith-Torgov total synthesis of 19-norsteroids.[9] The preparation and properties of 5,19-cyclosteroids was the subject of several studies.[10,11] Interestingly enough, the Villsmeier reaction on enamines of 3-keto-$\Delta^4$-steroids led to the 4,6-diformyl derivatives.[12]

Additional work with ammonium sulfide has shown that it is a useful reagent for the hydrogenolysis of selenides obtained as by-products in the selenium dioxide dehydrogenation of 3-keto-$\Delta^4$-steroids to the corresponding $\Delta^{1,4}$-ketones.[13]

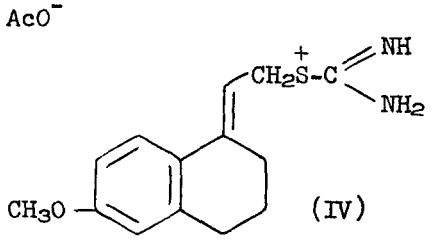
Among new compounds of interest one should mention 10$\alpha$-estra-4-en-3-ones,[14] A-homotestosterone,[15] and 2-oxa-estra-5-en-3-ones.[16]

A total synthesis of resolved 17$\beta$-hydroxy-des-A-androst-9-en-5-one has been described in connection with the preparation of 10$\alpha$-methyl steroids.[17,18] Conessine was degraded by an elegant and simple method to

13β-cyano-18-nor-5α-androstane derivatives.[19] The photolysis of 3-oxo-4,5-epoxides led to 3,5-dioxo-10(5→4)abeo steroids.[20]

Pregnane Series. Steroid allyl and propargyl enol ethers undergo the Claisen rearrangement.[21] Surprisingly, cyanogen bromide reacts with 18-hydroxy-20-dimethylamino steroids to form the corresponding 18,20-epoxy derivatives.[22] Additional work has been reported on the synthesis of 18-methyl steroids.[23,24,25]

Estratriene Series. The Wyeth chemists have published details of their work[26,27,28,29] on the total synthesis of estratriene derivatives prepared mainly for biological evaluation. In addition they described also a new total synthesis of equilin.[30] The Merck group working along similar lines has found that IV is easily prepared from III and can be used to alkylate 2-alkyl-cyclopentane-1,3-diones in cases where III fails.[31] They also

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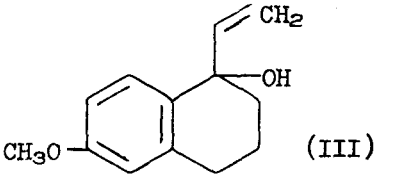



(III)

published work on the chemistry of dl-8(14)dehydroestrone.[32] The total synthesis of 6-thia,[33] 4-aza,[34] 6-aza,[35] and 8,13-diaza[36] steroids has been reported.

Along different lines, it was interesting to note the report that 15β-hydroxyestrone and 15β-hydroxy-17β-estradiol are present in the urine of pregnant women.[36]

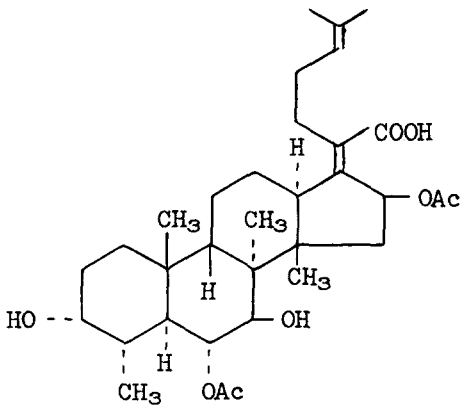
Corticosteroids. An interesting paper described the use of an iridium catalyst for the stereoselective hydrogenation of a 16-methylene steroid.[37] Some interesting theoretical considerations were discussed in connection with the solvolysis of 9α,11β-dichloro-steroids.[38] A novel reagent, sulfur dioxide and N-bromoacetamide, for the dehydration of 11β-hydroxy-steroids has been discovered.[39] The total synthesis of a hydrocortisone derivative was described by the Roussel group.[40]

Sterols. A considerable amount of work was devoted to the study of backbone rearrangements.[41,42,43] While partial syntheses of an insect moulting hormone, ecdysone, were announced by several groups,[44,45,46,47,48] evidence is accumulating on the widespread occurrence in nature of its hydroxylated derivatives. This year saw the isolation of 20-hydroxyecdysone[11] from silkworm[49] (ecdysterone),[50] crayfish[51] (crustecdysone),[52] oak-silk moth, tobacco hornworm,[53] and from plants such as Podocarpus nakaii,[54] Podocarpus elatus,[55] and Achyranthis.[54] The ready isolation of insect-moulting hormones from plants in contrast to the extremely poor

yield from insects and other sources makes it possible to obtain for the first time large amounts of active substances for biological experimentation.

Nitrilium ion intermediates have been shown to have synthetic utility.[56] Addition of phosphate carbanion to 3-keto steroids could be controlled to yield either cis or trans olefin.[57]

Antibiotics. Cephalosporin $P_1$ was shown to have structure V with a fusidane framework:[58]




(V)

Fusidic acid has been degraded to adrenocortical hormone analogs[59] and to derivatives of 4α,8α,14β-trimethyl-18-norandrostane.[60,61] The structure of Withaferin A has been confirmed by degradation to bisnor-5α-cholanic acid.[62]

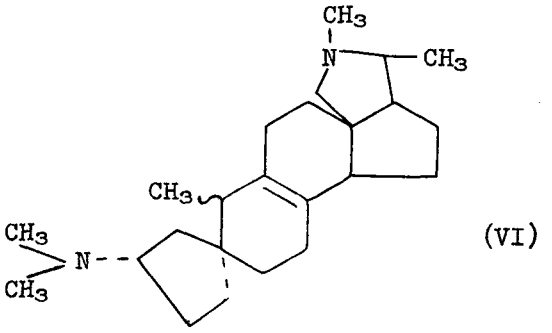
Cardenolides. The synthesis of digitoxigenin was announced,[63] while cardenolides of novel structure were described by the Ayerst chemists.[64] Reichstein and his group are continuing their work on the structure determination of various natural cardenolides.[65,66]

Steroidal Alkaloids. Photolysis of (22S:25S)-N-chloro-22,26-imino-5α-cholestan-3β-ol and the corresponding 16β-hydroxy substituted (22S:25R)-, (22S:25S)-, and (22-S:25R)-N-chloro compounds caused fragmentation to a mixture of the corresponding 20R and 20S chloropregnane derivatives. Whereas photolysis of (22R:25S)-N-chloro-22,26-imino-5α-cholestan-3β-ol gave the corresponding 16-chloro derivative.[67] UV-irradiation of the stereoisomeric N-nitroso-22,26-iminocholestane-3β,16β-diols in acidic solution led to the corresponding spirosolane alkaloids soladulcidine, solasodine, and tomatidine respectively, by way of the intermediate $\Delta^{22(N)}$

imine.[68]

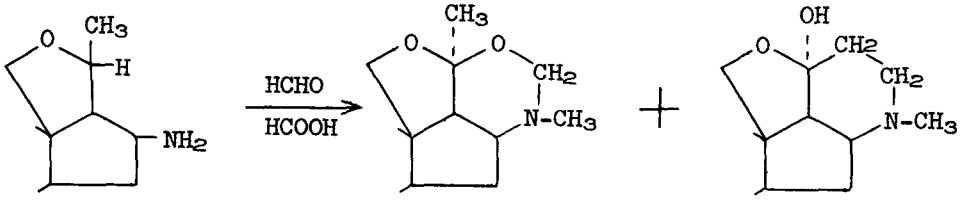
Dehydrogenation of Demissidine with mercuric acetate or N-bromosuccinimide gives the corresponding $\Delta^{22(N)}$ imonium salt as the main product.[69]

The acid catalyzed rearrangement of conessine was reinvestigated recently and the structures of the two reaction products isoconessine[70] and neoconessine[71] were elucidated. Isoconessine was proved to be 3β-dimethylamino-5β-methyl-19-norconan-8-ene whereas neoconessine has the novel structure 3α-dimethylamino-abeo-1(10→5)5α-conan-8-ene (VI).



(VI)

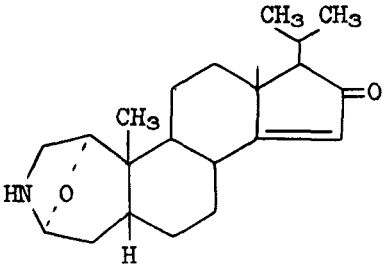
It is interesting to note that in the case of isoconessine, the double bond migration ends at carbon 8, whereas in the case of the cholesterol derivatives the process continues further causing rearrangement of the angular methyl attached to the 13 position.[41,42,43] Steroidal alkaloids from Paravallaris microphylla, containing the 18→20 lactone system, have been related to conessine, by the conversion of N-methyl-dihydroparavallaridine to dihydroconessine.[72] An unusual hydride transfer was discovered[73] during the Eschweiler-Clarke methylation of compound VII. The products isolated, VIII and IX, were derived from an intermediate oxidized at $C_{20}$.



(VII) (VIII) (IX)

Alkaloid A isolated from Sarcococca Pruniformis[74] is a 3α,20α-diamino-5α-pregnane derivative. Also possessing a pregnane skeleton are the various alkaloids isolated recently from the leaves of Holarrhena Antidysenterica.[75] In particular, it is interesting to note that kurchiphylline is 2α-hydroxy-3β-dimethylamino-5-pregnen-16-one.

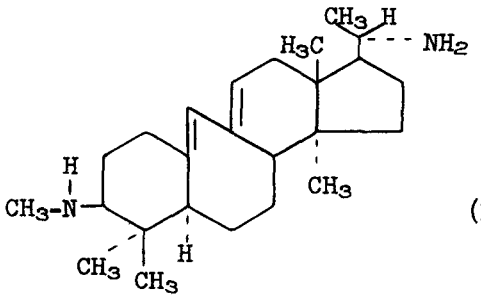
Samandenone, a new salamander alkaloid was isolated and identified[76] as X:




(X)

A review on salamander alkaloids has been published.[77]

Buxenine-G, a cytotoxic alkaloid isolated from Buxus sempervirens, was shown to possess structure XI, which was established unambiguously by x-ray analysis of Buxenine-G dihydroiodide.[78]




(XI)

The structurally related Buxpsiine,[79,80] also isolated from Buxus sempervirens, was proved to be 3β-dimethylamino-4,4',14α-trimethyl-19-nor-B-homo-pregna-9a(10),9(11),16-trien-20-one.

Work is continuing on the isolation and identification of new steroidal alkaloids from boxwood species, such as Buxus balearica,[80] Buxus malayana,[81] Buxus rolfei,[82] Buxus sempervirens,[83] and Buxus koreana.[84] With few exceptions, these alkaloids have an unusual skeleton containing a cyclopropane ring and are derived from the 4,4',14α-trimethyl-9β,19-cyclo-5α-pregnane system.

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# Chapter 31. Reactions of Interest in Medicinal Chemistry

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An ever increasing number of new and/or novel reactions makes an interesting chore of selecting reactions for this chapter. This report will cover only a small number of the unique reactions reported during 1966 but will attempt to select representative examples of high utility to the medicinal chemist.

Reports of excellent results utilizing soluble catalysts continue to support the superiority in selective reductions of systems containing multiple double bonds. Tris (triphenylphosphine) rhodium chloride as a soluble catalyst for hydrogenation allows the reduction of ω-nitrostyrene to phenylnitroethane and cinnamylchloride, in part, to phenylpropyl chloride. Deuterium is added to a double bond without the introduction of additional labeling.[1,2] Aluminum hydride has given superior results in the reduction of amides, nitriles, oximes, isocyanates, and Schiff bases to the corresponding amines.[3] A comparison of its reducing characteristics with those of lithium aluminum hydride has also been reported.[4] The eneamine of cyclooctanone is reduced to cyclooctane in 85% yield with aluminum hydride.[5]

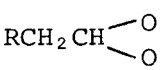
Hydrogenation of α,β-unsaturated aldehydes on Raney zinc catalysts was studied. Saturated aldehydes were obtained along with saturated alcohols. The amounts of each were dependent on reaction conditions. On reduction of nitriles and oximes with Raney alloy improved yields of primary amines were obtained.[7] Treatment of ketoximes with Raney-nickel yields the ketones.

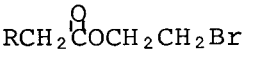
The reduction of nitrobenzene with cyclohexane, cyclohexene, and n-hexane over a series of catalysts on aluminum oxide afforded aniline in yields greater than 90%.[8] An iridium catalyst prepared by Adams' method is useful for the reduction of nitroaromatics to hydroxylamines.[9]

Although α,β unsaturated acids are not usually reduced by aqueous sodium borohydride, they are in the presence of cyanocobalt complexes to yield the saturated acid.[10] Various cyanostilbenes were reduced to the corresponding diphenyl cyanoethanes with sodium borohydride in tetrahydrofuran; yields ranged from 72-98%.[11] A tetrahydrofuran solution of diborane reduced oximes to the corresponding alkylhydroxylamines.[12] A discussion of the stereoselectivity in the reduction of ketones by metal hydrides gives an excellent route to axial alcohols.[13]

Hueckel and co-workers indicate three methods by which reductions in liquid ammonia (Birch reduction) can be performed. They show that the reduction is substantially affected by the sequence in which reagents are added.[14]

Mechanisms of dimethylsulfoxide oxidations have been studied and an explanation is now available for the fact that the oxidation proceeds in a weak acid medium and is sensitive to pH change.[15a,b] A new reagent for the oxidation of alcohols to ketones in neutral solution at room temperature is 4-phenyl-1,2,4-triazoline-3,5-dione. Dry benzene is a convenient solvent, from which the phenylurazole separates during the reaction.[16] Cyclic acetals can be oxidized to bromine containing esters under mild conditions using N-bromosuccinimide.[17] Palladium acetate gives mainly enol acetates with terminal





olefins and mainly allylic acetates with 2-olefins.[18] Cobalt (II and III) acetates and sterates catalyze the allylic oxidation of cyclohexene by oxygen at 60° in acetic acid. Molecular oxygen can also be utilized for the direct epoxidation of olefins in acetone solution[20]

The reaction of propene and other olefins with mercuric salts of some strong oxy acids was shown to give acrolein and analogous carbonyl compounds. This reaction proceeds through a 1:1 adduct which in aqueous solution is probably of the form $CH_3CH(OH)CH_2Hg^+x^-$.[21] Inclusion compounds of diperoxycarboxylic anhydrides with urea have been prepared as peroxyacid precursors.[22] The inclusion compounds are not shock or heat sensitive.

Various unique replacement reactions in aromatic systems have been reported. A one step replacement of aromatic amino groups by chlorine or bromine involves treatment with $CuCl_2$-NO or $CuBr_2$-NO complexes. The complex is formed *in situ*.[23] Nitric oxide acts as a diazotization agent and can be used to give higher yields than obtained by other methods.[24] Another method of aromatic chlorination involves desulfonylation of aromatic sulfonyl chlorides utilizing tris(triphenylphosphine) rhodium chloride[$(Ph_3P)_3RhCl$].[25] Arylsulfonyl azides give direct amination of benzene when the latter is treated with the azide in the presence of $H_2SO_4$.[26]

Hydroxyl groups can be replaced by methyl at primary carbon atoms via the sodium salt of dimethyl sulfoxide utilizing the following sequence:[27]

$$ROTs + NaCH_2SOMe + Me_2SO \longrightarrow RCH_2SOMe + Ni/H \longrightarrow RMe$$

The hydrogenator previously described, which generates hydrogen automatically from $NaBH_4$ has been adapted to hydrochlorination of reactive alcohols and olefins. The apparatus allows quantitative conversion into product without excessive contact with hydrochlorination agent.[28] Phosphorous

acid with iodine and alcohols affords alkyl iodides in 75-96% yield.[29] In polar inert solvents, $CuCl_2$ can convert acid chlorides, anhydrides, and carboxylic anhydrides into α-chloro derivatives with selectivities greater than 95%. This method can be used to prepare pure α-monochloro acids regardless of the number of α-hydrogen atoms in the substrate.[30]

A simple method for the preparation of primary amines from aliphatic halides involves the treatment of the halide with KOCN in alcohol to give the urethane which is hydrolyzed to the amine. In general the yields are high.[31] A novel conversion in which tetrakis(dimethylamino) titanium converts carboxylic acids and their common derivatives in orthoamides and ketene N,N-acetals in good yields is described.[32a,b]

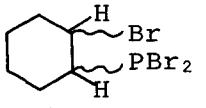
Quinoline 1-oxide can be substituted by mercaptans with the 3-isomer being the major product.[33] Heterocyclic acid amides, such as 10-methyl-9-acridone and 2-methyl-1-phthalazone, were aminoalkylated by $ClMg(CH_2)_3NMe_3$. The carbinols formed have the character of pseudo bases and were transformed to the corresponding acridinium or phthalazinium salts.[34]

A useful synthesis of a wide variety of ketones was developed by mono- and dialkylation of β-oxosulfoxides. Reductive cleavage of these alkylated products with aluminum amalgam produces the desired ketone[35] Hauser and co-workers continue to investigate the alkylation of dianions.[36] A study was made of the relative reactivities of disodio salts of β-diketones toward alkyl halides in liquid $NH_3$. The order of relative reactivities observed was isobutyryl, propionyl> acetyl > phenylacetyl. Sodium α-sodioacetate reacts with aliphatic dihalo compounds and aromatic hydrocarbons containing side chain halo groups to give the di- and monocarboxylic acids, respectively.[37] O-alkylation of ketones to produce enol ethers utilizing triethyloxonium fluoroborate or a dialkyl sulfate occurs readily in dimethylformamide and dimethyl sulfoxide.[38]

1,3-Dipolar addition to unsaturated compounds affords routes to unique and interesting structures. The 1,3-dipolar cycloaddition of the O-methyl ether of dinitromethane to methyl acylate produced N-methoxy-3-nitro-5-carbomethoxy-isoxazolidine in 65% yield. Similar results were obtained with other olefins.[39] Acetonitrile N-oxide adds to enynes to form isoxazolines and isoxazoles.[40]

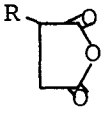
Additions to multiple bonds catalyzed by radical initiators produce novel synthetic methods. Carboxylic acids add to acetylenic compounds in the presence of organic peroxides. Acetic acid and acetylene yield adipic acid; 1-hexyne and acetic acid give octen-3-oic acid.[41] Radical amination of olefins with hydroxylamine sulfonic acid and hydroxylamine in the presence of $FeCl_2$ gives the corresponding 1-amino-2-chloro compounds.[42]

The steric course of additions of amines and alcohols to acetylene dicarboxylic ester was studied under various conditions.[43] Secondary amines gave stereospecifically cis addition product. Nitrosyl formate can be generated in situ in the presence of an olefin to yield the nitrosoformate which on hydrolysis affords the hydroxynitroso compound in high yield.[44] Transoximination gave the α-hydroxy ketone. Phosphorous tribromide can be added to olefins to yield 1:1 adducts with uv irradiation, peroxides, or heat as initiators.[45]




Similar results are obtained with $PCl_3$. Maleic anhydride condenses with olefins in an ene synthesis to give substituted succinic anhydrides.

An review of the preparative use of the Diels-Alder

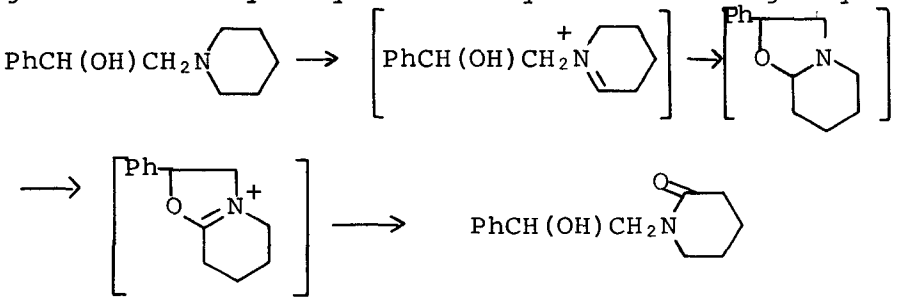



reaction relating predominantly to recent results gives a systematic approach to this useful reaction.[47] The direct brominative cyclization of methyl farnesate was accomplished utilizing N-bromosuccinimide in aqueous $MeOCH_2CH_2OMe$.[48]

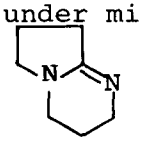
Borontrichloride[49] and boron tribromide[50] have been studied further as dealkylating agents with regard for specificity and neighboring group effects.

Mercuric salts of carboxylic acids decompose in uv light or in the presence of peroxides to form alkylmercury derivatives.[51] Aqueous peroxydisulfate can be used to decarboxylate N-acetylamino acids to give aldehydes or ketones, acetamide, and $CO_2$.[52]

Dehydrogenation of amine derivatives with Hg-EDTA can give rise to hydroxy lactams by the following sequence:[53]



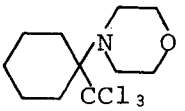

A reagent for dehydrohalogenations is 1,5-diazabicyclo(4.3.0)-5-nonene has been shown to be versatile and capable of being used under mild conditions.[54] The reaction of vic-dibromides

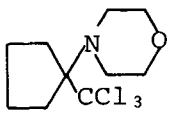



with thiourea gave olefins in high yields.[55] The ylides, alkylidenetriphenylphosphoranes, tend in general towards cis olefin formation. The proportion of cis olefin is highest when ylide is salt-free while lithium salts favor trans isomers.[56] Ylides can be prepared by the treatment of

phosphines $R^1R^2PCH_2R^3$ ($R^1$=Ph; $R^2$=Ph, $CH_2Ph$; $R^3$=Ph, $CO_2Et$) with activated olefins $H_2C=CHR^4$ ($R^4$=CN, $CONH_2$, $CO_2Et$). These ylides can be used in situ in Wittig olefin syntheses under mild conditions, with or without a hydroxylic solvent.[57] The synthesis of stable sulfonium ylides is described.[58]

Trichloroacetic acid reacts with cyclic enamines to give the chloromethyl derivatives. This demonstrates that trichloromethyl anion will react preferentially with an organic cationic species rather than undergo loss of chloride ion to give dichlorocarbene.[59]



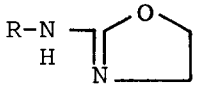

Oxidation of benzyl ketone hydrazones with $Hg_2$ ($O_2CCF_3$) in ether or dioxane gives a direct synthesis of phenylacetylenes ($C_6H_5C{\equiv}CR$).[60] Olefin formation occurs with saturated aldehydes and carboxylic acids when they are allowed to react with ruthenium and rhodium complexes.[61] Olefins can be converted to aldehydes in high yields by ozonization followed by treatment with dimethylsulfide.[62]

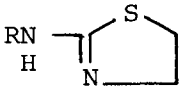
Dimethylsulfoxide was found to increase the rate of hydrolysis in esters which were resistant to saponification.[63] Treatment of alkyl toluenesulfonates with sodium naphthalene anion radical in tetrahydrofuran constituted an almost ideal procedure for regenerating the corresponding alcohols.[64] The hydration of nitriles to amides in the presence of nickel catalysts shows an increase in yield with the addition of pyridine.[65]

A convenient synthesis of tert-alkyl amines involves the amination of the methine group with trichloroamine-aluminum chloride.[66] Organoboranes from both hindered and unhindered

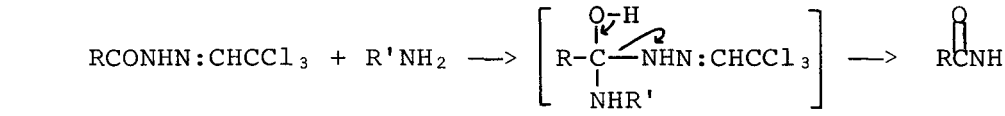
$$R_3CH + NCl_3 + AlCl_3 \longrightarrow R_3CNH_2$$

olefins can be converted to the corresponding amines by treatment with $H_2NOSO_3H$.[67] Isocyanates and isothiocyanates give 2-oxazolinyl-2-amines and 2-thiazolinyl-2-amines when treated with aziridinium tetrafluoroborate.[68] A continuing investigation of amino ketone rearrangements has produced a novel route to a variety of 2-alkylamino-2-phenylcyclohexanones and 2-alkylamino-2-phenylcyclohexanols.[69,70] 2-Alkyl-Δ'-piperidienes were obtained in good purity from the corresponding 1-alkylcyclopentanols with $NaN_3$ and $H_2SO_4$.[71] Primary, secondary, and tertiary amines were produced by the reaction of nitro compounds with aliphatic alcohols in the presence of aluminum oxide.[72]




The formation of various amides from aliphatic and aromatic carboxylic acid hydrazides in the presence of chloral involves a novel dehydrazination reaction.[73] Reductive



elimination of diazo nitrogen via the sequence Y:N:N ⟶ $YH_2$ + $N_2$ (Y=RCON:, RCOC:) can be extended to include reductive desamination of primary amines through the diazonium salt.[74] Benzazide on photolysis in isopropyl alcohol gave quantitative yields of benzamide and acetone. Hydrazines can be acetylated in the presence of dicyclohexylcarbodiimide to give monoacetyl (on the more basic nitrogen) and diacetylhydrazines in good yields.[76]

Glycidic nitriles can be prepared from chloroaldehydes by treatment with sodium cyanide.[77] RR'CClCHO + NaCN ⟶

Refluxing 1-nitro-2-alkanols in HCl gave high yields the corresponding α-hydroxy acids.[78] Ketol nitrates (e.g. p-Br-$C_6H_4COCH_2ONO_2$) are readily converted in good yields to the corresponding dicarbonyl derivatives (e.g. p-$BrC_6H_4COCHO$ under the influence of sodium acetate in $CH_3SOCH_3$.[79] The α-bromo ketones are treated with silver nitrate to give the nitrate which is used without isolation.

The efficiency of drying agents of organic solvents was examined using near IR techniques. The agents $CaCl_2$, $CaSO_4$, $MgSO_4$, mol. sieves-type 4a, and $Na_2SO_4$ were determined for the removal of $H_2O$ in solution from $C_6H_6$, $Et_2O$, and EtOAc. $CaCl_2$, $CaSO_4$, and mol. sieves were fast drying; $MgSO_4$ and $Na_2SO_4$ were slow drying agents and removed only small amounts of $H_2O$.[80]

In small amounts methyl sulfinyl carbanion is prepared safely. When 4.5 moles of sodium hydride was added in 5 portions to 18.4 moles of dimethylsulfoxide at 70° and stirred the temperature rose sharply and an explosion occurred.[81]

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# Chapter 32. Antiradiation Agents

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Advancement in knowledge of structural requirements for chemical protection against ionizing radiation has continued mainly through modification of structures having known protective effects. Knowledge of the effects of ionizing radiation on biological systems, particularly in regard to enzymes, proteins, and nucleic acids, has undergone a marked expansion, and new approaches to improved radioprotective agents should be forthcoming. Since this discussion is concerned primarily with the development of new protective agents for mammalian cells, mention here can be made only to several recent reviews concerned with radiobiological effects of interest to the medicinal chemist. These include the radiation chemistry of proteins and enzymes[1]; electron spin resonance and the effects of radiation on biological systems[2]; and the chemical, physical, and biological aspects of energy transfer in radiation processes[3]. Chapters on ESR studies, radical scavengers, and serotonin in regard to radioprotection[4] have been written as well as one on the reactions of the hydrated electron with biological substances of importance[5].

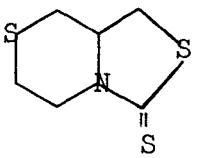
Reviews - A handbook of radioprotective agents has appeared in Russian[6], and a chapter on chemical agents that influence the effects of radiation in mammals is now included in "Progress in Drug Research"[7]. Radioprotection of mammals by thiolic compounds[8] and aminothiols[9,10] has been reviewed, as well as the more general aspects of chemical radiation protection[11,12]. Proceedings of the Second Symposium on Protection against Radiations in Space has been printed[13]. The role of oxygen in chemical radioprotection has been discussed[14], as well as the conversion of protective drug precursors to protectors *in vivo*[15]. A bibliography on antiradiation drugs covering 1959-1966 is available[16].

Radioprotection of Mammals - Chemical modification of the MEA structure continues to be worthwhile in the synthesis of new radioprotective agents, and some highly active derivatives have been reported. Substitutions on either carbon, sulfur, or nitrogen have provided active products, and introduction of other functional groups, generally containing sulfur, has also been made. In a few examples, including the condensation product of carbon disulfide with cysteine, an improvement over the toxicity of MEA or MEG has been found. An increased amount of antiradiation testing has been observed for natu-

rally-occurring compounds, and those compounds known to be physiologically active.

Amino thiol derivatives. In a series of N-monosubstituted 2-mercaptoethylamines, the β-phenethyl derivative gave good protection to mice vs. 1000 r, and the β-2-thienylethyl derivative gave fair protection.[17] No protection was shown by N-monosubstituted derivatives containing thioureido groups;[18] and no protective activities were reported for N-monosubstituted Bunte salts of MEA carrying sulfone substituents.[19] Several 2-amino-2-alkyl-1,3-propanedithiols failed to show significant protection in mice, but L(+)-3-amino-4-mercapto-1-butanol gave good protection.[20]

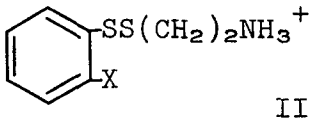
N,N'-Polymethylene bridging of the MEA molecule, giving $XS(CH_2)_2NH(CH_2)nNH(CH_2)_2SX$, removed protective activity in mice where X was $SO_3H$, but provided good protection where X was $PO_3HM$ and n=3 or 4.[21] Good protection vs. 1000r in mice was also reported[22] for the following MEA derivatives: S-2-amino-2-methylpropylthiosulfuric acid (the N-decyl derivative was active vs. 750 r), S-2-amino-2-methylpropylphosphorothioic acid (the corresponding thiol was previously reported inactive), 2-amino-1-pentanethiol, and 2-amino-3-methyl-1-butanethiol. S-2-amino-1-pentylphosphorothioc acid showed fair protection, and the cyclic dithiocarbamate I gave good protection. Reaction of sultones with MEA and several thiones provided sulfonic acid inner salts, of which $HS(CH_2)_2\overset{+}{N}H_2(CH_2)_n\overset{-}{S}O_3$ and its disulfide (where n=3) were protective in mice vs. 1000r.[23] Corresponding butane derivatives (n=4), Bunte salts (n=3,4) and dithiocarbonates, $\overset{+}{N}H_3(CH_2)_2SC(=O)-S(CH_2)_n\overset{-}{S}O_2$, were inactive, as well as were several isothiuronium sulfonates and heterocyclic sulfonates, which points to the same high degree of specificity as shown by other charged derivatives of MEA.



I

Synthesis of the thiolsulfinate of MEA, $NH_2(CH_2)_2S(O)S-(CH_2)_2NH_2 \cdot 2HCl$, has been claimed;[24] as well as the preparation of 2-aminoethaneselenic acid[25]; no testing results were reported. A patent on 1-phenyl-2-aminoethanethiols as radiation protectors has appeared.[26] A comparison of the protective effects in mice vs. 200-1000 rad of MEA and the Bunte salts of MEA, MPA, and N-substituted derivatives has been made.[27] The Bunte salts were claimed to give greater protection and possess about half the toxicity of MEA. A bisthiolsulfonate of N-acetyl MEA, $AcNH(CH_2)_2SO_2S(CH_2)_4SO_2S(CH_2)_2NHAc$, was found to be non-protective in mice.[28]

Unsymmetrical disulfides of MEA of type II were found active in mice vs. 1000 r, the most promising being zwitterions.[29] Good protection was given where X was $\overset{-}{C}O_2$ or $\overset{-}{S}O_3$ and where the 2-aminoethyl group was replaced by 2-pyridylmethyl.

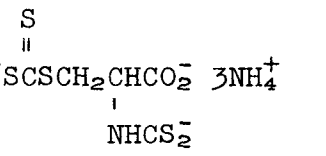


II

No correlation was found between the nature of X, antiradiation potency, or resistance of the disulfides to disproportionation by either thermal or photochemical means. Where X was located *para*, no protective activity was shown.[30]

Cyclic analogs of AET were examined in an attempt to find relationships between free SH content of the compound and reductive capacity, inhibition of tissue respiration, and radioprotective effect.[31] Where AET and APT gave 80-100% protection to mice *vs.* 730 r, alkylisothiourea derivatives had only a moderate effect (35-50%), and aminoalkylmercaptoimidazolines gave only 20-30% protection. It was concluded that inhibition of tissue respiration does not depend on SH content, and that release of SH, reductive capacity, and inhibition of tissue respiration determine toxicity rather than protective ability.

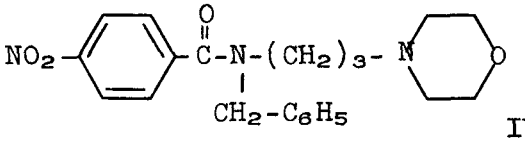
*Other sulfur compounds.* Several dithiocarbamates containing disulfide functions were non-protective in mice, as well as the S-benzyl dithiocarbamate of ethylenediamine.[32] The S-trithiocarbonate N-dithiocarbamate of cysteine, III, however, was found to give the same order of protection to mice *vs.* 800-1100 r as AET and was 2-3 fold less toxic than AET.[33] It is believed to be converted *in vitro* to the dithiocarbamate of cysteine, a compound not yet synthesized. A series of antibacterial sulfonamides showed varying degrees of protection in mice *vs.* 800 r, the best effects being shown by sulfanilamide (30% survival), sulfathiocarbamide (45%), and sulfamethazine (60%).[34]



III

*Heterocycles.* A series of N-alkylthiazolinium salts proved to be inactive in mice;[17] as was also a series of 2-aminothiazolines;[35] probably due to slow rates of hydrolysis to aminothiols. Various salts of 2-aminothiazoline showed varying degrees of protection in mice *vs.* 1090 r, the most effective being the succinate, fumarate, benzoate, and glutamate.[36] Protection was somewhat less than that afforded by AET. Mice were protected (25% survival) *vs.* 800 r by 1-(p-ethoxyphenyl)-4-(D-arabinotetrahydroxy)butylimidazoline-2-thione.[37] A series of s-triazoles, mesoionic s-triazoles, and 1,3,4-oxadiazolium salts was screened in mice *vs.* 900 r without report of significant protection.[38]

*Physiologically-active substances.* In a series of p-amino and p-nitro derivatives of procainamide tested in mice *vs.* 880 r, none of the amino derivatives were protective, but several p-nitro compounds were.[39] Compound IV provided 100%



IV

survival. Among 14 organo-phosphorus compounds designed to form reversible antienzyme-enzyme complexes and thus protect cholinesterase, only 2 (both thiophosphate esters) showed protective activity in mice vs. 750-800 r.[40] No relation of anticholinesterase activity to radioprotective ability was found. 2-Aminoethylphosphoric acid was claimed to protect mice, however.[41]

Psychotropic drugs recently reported to be radioprotective include imipramine;[42] taractan, valium, tryptizol, and insidon.[43] Taractan provided 73% survival in mice vs. 800 r. Substances known to stimulate turnover of NADP·$H_2$, a physiological reducing agent, showed significant protection in mice vs. 900 r.[44] These included 4-hydroxybutyric acid and 6-phosphogluconolactone.

Naturally-occurring substances. Significant radiation protection has recently been claimed for complamine, a nicotinic acid derivative;[45] Ca pantothenate;[46] leucodelphinidine;[47] tea catechols and a gallatetannin complex;[48] isopropylarterenol;[49] tyramine;[50] and methoxamine.[50] Among a group of commonly used antibiotics, the tetracyclines gave the best survival rate, which was attributed to an increase in metabolic activity.[51] Cyanocobalt-chlorophyllin has been patented as an antiradiation drug;[52] and several articles have reported radiation protection from DNA, RNA, and derivatives distinct from post-irradiation repair therapy.[53-55] Other substances reported to show radioprotective effects include uridine but not cytidine monophosphate;[56] polysaccharides extracted from typhoid and Proteus organisms;[57] and typhoid-paratyphoid vaccine.[58]

Radiosensitizers - The role of halogenated thymidine analogs in inducing radiosensitization of cells has been reviewed.[59] The common thiol-binding reagents sensitized mice to whole-body irradiation of 525 or 810 rads;[60] possible by removing endogenous protective thiols. Sensitization of mice or rats was also reported for nembutal;[61] nalorphine;[62] organic peroxides;[63] hematoporphyrin;[64] riboflavine (increased by K);[65] and cupric salts.[66] The sensitizing action of cupric salts was prevented by thiols. Sensitization of ascites lymphosarcoma NKLy cells was reported for ambunol, an aminophenol.[67]

Radiosensitizing effects in bacteria have been found among phthalanilides, phenaziniums, and isoindoliniums.[68] Hadacidin;[69] vitamin $K_5$;[70] chloral hydrate and other halides (reversed by MEA and cysteine);[71] and organic nitroxides[72] have also sensitized bacteria to ionizing radiation. A direct relation between ability to bind p-hydroxymercuribenzoate to

thiols among several species of bacteria and radiosensitivity has been observed.[73] The sensitizing action of iodoacetamide on enzymes has been stated not to be the result of reaction with thiol groups, however.[74]

Radioprotective Drugs and Radiosensitizers in Radiotherapy of Tumors - Repeated administration of methyl ethyl ketone peroxide increased the therapeutic effect of X-rays on lymphosarcoma in mice and rats,[63] possibly be decreasing catalase activity. Irradiation (2750 r) of transplanted rhabdomyosarcoma in mice gave complete remission in the presence of hematoporphyrin and its copper complex.[75] Cysteine thiosulfate protected organs but also exerted some protection on Crocker sarcoma in mice, thus diminishing the therapeutic effect.[76] The mitotic activity of MEA, AET, and serotonin in normal and malignant cells has been related to their protective effects.[77]

Potentiating effects on irradiation of various tumors have been reported for actinomycin D,[78] combination of cyclohexanol succinate and lysozyme,[79] 6-chlorothymine,[80] heterologous RNA,[81] and menadione.[82] No protection was shown in experimental tumors by MEA, thiourea, serotonin, or iodoacetate; protection was given by 6-aminonicotinamide and menadiol diphosphate, however.[83]

The distribution of AET in the organs has been observed; tumors showed the lowest concentration.[84] Cysteamine-S-phosphate was found to give significant concentrations of MEA in 13 of 15 tissues observed, including tumor tissue,[85] whereas its methyl ester remained uncleaved in vivo.

Modes of Radioprotection - The major hypotheses of the modes of radioprotection, e.g., hypoxia, radical scavenging, and mixed disulfide formation, continue as the basis for much experimentation, but a very complex picture is emerging for the phenomenon of radioprotection. For the amino thiols, at least, free radical formation, oxygen, metal ions, energy transfer, and possibly mixed disulfide formation may all be involved. Bacq, however, favors a "biochemical shock" mechanism for the amino thiols, in which fixing of the thiols to important macromolecules of the mitochondria liberates certain substances, possibly enzymes, which results in inhibition of carbohydrate utilization.[86]

In regard to anoxia or hypoxia as a major mechanism, most recent evidence has been unfavorable, particularly for the action of amino thiols. For instance, a correlation was observed between radioprotective activity and depressive effect of thymidine uptake in DNA synthesis, whereas anoxia did not inhibit the uptake.[87] No relation was shown between radioprotective activity and ability to increase oxygen uptake in spleen, kidney, muscle, or red blood cells,[88] and the intracellular oxred state in rats was unaltered even after massive doses of radiation.[89] Amino thiols, nitrite, and PAPP did not affect

respiration of brain or liver mitochondria in either healthy or irradiated rabbits, but did in liver mitochondria *in vitro*.[90] Post-irradiation exposure of rats to respiratory inhibitors, however, did reduce mortality and prevent loss of nuclear structure from thymocytes.[91]

Evidence for the activity of some radioprotectors as inhibitors of free radical processes has appeared, and the subject has been reviewed.[92] Involvement of MEA,[93] as well as of metal ions,[94] in free radical formation in proteins and bacteria has been observed. It was also found that cysteine and glutathione could accept electrons from irradiated proteins, whereas cystine and non-sulfur compounds did not.[95] Presence of metal ions, particularly cupric, had a protective effect for ribonuclease, presumably by intercepting electrons and preventing radical formation on the enzyme.[96] A protective effect of mucopolysaccharide polyanions and cysteine for trypsin and RNA, however, was not considered to be due to transfer of radiation energy to the protectors.[97] Furthermore, substances known to react with H atoms or the aqueous electron did not protect hydrated E. coli cells from X-rays.[98]

Further examples that some radioprotectors cause an increase of endogenous thiol groups in various tissues have been reported during the past year. Amino thiols, cystamine, serotonin, compounds which lower $O_2$ tension in tissues, and anoxia, bring about an increase in cellular thiol content, which, with the amino thiols, is 30-40 fold greater than that supplied by the protective agent.[99] This increase was also observed with diethyl dithiocarbamate, but not for disulfiram, its disulfide, which is also not protective.[100] Decrease in both protein- and non-protein-bound thiol content has been observed with increasing doses of radiation.[101]

Evidence for the binding of protective molecules to important cellular macromolecules continues to appear. The protection of GED against inactivation of trypsin, lysozyme, and aldolase was considered not due to radical scavenging, but to mixed disulfide formation.[102] Protection of DNA by diamino disulfides was attributed to bound disulfide,[103] and protection of lactate dehydrogenase[104] and catalase[105] by serotonin was also attributed to complex formation, possibly with the metal ions in the enzymes.

Further indications of the importance of metal ions in radiation damage and protection have been found. Radiation death of L. delbrueckii, found to be due to the $H_2O_2$ formed, was prevented by catalase, as well as by EDTA, indicating that oxidation by $H_2O_2$ is catalyzed by metal ions.[106] Radioprotective properties of heavy metal ions, particularly $Fe^{+2}$ and $Fe^{+3}$, and MEA for plants was observed.[107] $Cu^{+2}$ was sensitizing, as it was for radiolytic deactivation of α-amylase and catalase.[108] Radiation-induced oxidation of cytosine and uracil in presence of

$O_2$ produced radicals, but in the presence of $Cu^{+2}$ and $Fe^{+3}$, radicals were not formed.[109]

Whereas radical formation appears to be a major role for the amino thiols in radioprotection, protein binding or reaction with metals probably occurs as well. At least, involvement in these reactions provides some basis for the necessity of the amine function, and for the high degree of structural specificity known for protection by amino thiols, which are not explained by radical trapping alone.

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# Chapter 33. PHARMACEUTICS AND BIOPHARMACEUTICS

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Basic investigations and developments in both pharmaceutics and biopharmaceutics area continue with increasing vigor. Although the bulk of published works still originates from academic institutions, an increasing proportion appears to be contributed by industrial organizations. General developments during the past year in these fields are summarized below.

## THERMODYNAMIC AND EQUILIBRIUM RELATIONSHIPS

The tendency of polyethylene glycol (PEG) to form addition complexes with iodine was reported again by Hiskey and Catwell.[1] Unfortunately, the authors were apparently not aware of a more extensive study of similar systems carried out ten years earlier[2] which suggested formation of at least two types of adduct involving PEG, $K^+$, $I^-$ and $I_2$. The more recent report differs in part from the earlier work in that PEG-$I_2$ complex containing $I_2$/monomeric unit ratio of one is suggested among others.

Bates, Gibaldi and Kanig[3] have studied the relative solubilizing tendencies of four bile salts with respect to hexesterol, griseofulvin and glutethimide. The effect, which is ascribed entirely to micellar solubilization, was determined at several temperatures and at salt concentrations up to 0.6M. The most marked effect was noted with hexestrol and least for glutethimide. Connors and Mollica[4] have compared the theoretical relationships of stability constants obtainable by solubility and other methods.

Chelation equilibria involving five different tetracyclines with cupric copper have been examined by Benet and Goyan.[5] These interactions appear to form essentially 2:1 (ligand:metal) complexes, the reaction showing large positive entropy changes.

## DRUG STABILITY

The past year was marked by relatively little activity in this area. Wadke and Guttman[6] reported on the base induced degradation of 9-methylisoalloxazine under both aerobic and anaerobic conditions. The initial product formed appears to be a carbinol amine. Finholt et al[7] have continued their investigation of anaerobic hydrolytic degradations of ascorbic acid with a study on the effect of metallic ions on the over-all rate. Although the reaction appears to be accelerated by doubly and triply charged species, the effect was surprisingly small.

## HETEROGENEOUS SYSTEMS

Powders, Suspensions, and Emulsions - One of the most significant articles in recent years is that by Hiestand[8] on particle-particle interaction in powders. The author has brought together much information from pharmaceutical research and from other disciplines and has perspectively assembled a rational treatise. The importance of plasticity and the true area of contact is clearly described. In addition, several novel methods for evaluating cohesion and adhesion of powder particles are described in this paper.

Nash and Haeger[9] have described the applications of the Zeta-Meter, a device which conveniently measures the electrophoretic mobility of suspension particles. The instrument appears to be useful in suspension and emulsion formulation work and in basic studies related to dispersed systems.

Mima and Kitamori[10] and Groves[11] have employed the Coulter Counter in studying the aggregation behavior of emulsions. This instrument continues to be useful for work of this type. Ho[12] has described a modification of this instrument which involves the simultaneous sorting of the particles into 400 size ranges. This improvement significantly extends the applicability of this technique.

Drug Release Rate Behavior - Desai et al[13] have reported results of extensive studies on the release of drug from matrices. Taking the basic physical model approach and beginning with the Higuchi relationship[14] these authors have quantitatively investigated the influence of many factors upon the rate of release. The equation was also found by Lapidus and Lordi[15] to apply to a system having hydrophillic gum as the matrix.

Goldberg et al[16] have continued their extension of the Japanese work[17] on utilizing melts to increase dissolution

rates of drugs. Succinic acid as well as urea was used as the vehicle in these studies. Since at elevated temperatures the dicarboxylic acid readily cyclizes to form succinic acid, some of the results should, perhaps, be re-examined.

## BIOPHARMACEUTICS

The pharmacological activity, efficacy, and toxicity of an administered medicament may be profoundly affected by the physico-chemical properties of the drug and the drug dosage form. Thus, such parameters as solubility, particle size, diffusional characteristics, availability and rate of dissolution of the drug have been the areas emphasized in most biopharmaceutical studies. The effect of these parameters on drug absorption is the subject of this review.

Diffusional Characteristics - The everted intestinal sac technique was employed by Aguiar and Fifelski[18] to quantitate the absorption of flufenamic acid. These workers determined the rate of permeability of flufenamic acid through the intestinal wall of the golden hamster, in vitro, and interpreted their results in terms of Fick's first law of diffusion. They observed a linear relationship between the concentration of the drug employed and the rate of intestinal permeation, thus establishing that the transport of flufenamic acid through the gut was by passive diffusion. By varying the pH of the external (mucosal) media, they found that the rate of permeation was inversely proportional to the degree of ionization, thus supporting the hypothesis that only the unionized moiety permeates the biological membrane.

While most drug systems evaluated have shown passive diffusional characteristics, Levy and Jusko[19] have reported that the oral absorption of riboflavin in man possesses characteristics indicative of specialized transport mechanisms. The evidence in support of such a conclusion was based on the finding that as the dose of riboflavin was increased, the per cent absorbed (based on urinary recovery) decreased. This effect was observed only in fasted patients and was not observed when riboflavin was administered with a meal. The authors suggest that the presence of food in the alimentary tract delays the transit time of riboflavin, thus keeping it in the presence of absorptive sites for longer periods of time.

In an interesting study utilizing tritiated mineral oil, Ebert, Schleifer, and Hess[20] have shown that contrary to previous opinions, a small but significant amount of mineral oil is absorbed from the alimentary tract following oral

administration. Their findings, employing doses consistent with those usually utilized for laxative purposes in humans, indicated that approximately 1.5% of the administered dose was absorbed unchanged with an additional 1.5% appearing in the carcass of the animals but arising from non-mineral oil sources. The origin of the non-mineral oil sources was not proved but may have arisen from exchange reactions prior to or after absorption or from non-polar metabolic products of mineral oil metabolism. The authors noted that once absorbed, the mineral oil was slow in leaving the body. The elimination curves illustrated a dephasic characteristic, the more rapid component resulting in a reduction of the mineral oil content of the body from 1.5% of the administered dose down to 0.3% in approximately two days. The slower component of elimination reduced the content further down to 0.1% in 21 days. In agreement with studies previously reported,[21] Ebert et al found that emulsification of the mineral oil greatly facilitated the oral absorption of mineral oil.

Although a mechanism to rationally explain their findings is not presented, Green et al[22] have reported data substantiating their previous observations on the influence of cholinesterase inhibitors on the oral absorption of sulfonamides.[23] These workers found that the four-hour plasma levels of sulfacetamide, sulfonilamide and sulfaguanidine were enhanced when the rats were pre-treated with neostigmine as compared to those plasma levels found in the controls. The earlier studies had indicated that this enhancement was not blocked by atropine, thus minimizing the possibility of an increased blood flow at the absorption site as being the mechanism for enhanced absorption. While perhaps not representing a major factor in the absorption of drugs through the intestinal membranes, the role played by cholinergic systems is worthy of further elucidation. It would be desirable, in this regard, to ascertain whether or not other drug systems are similarly affected by inhibitors of cholinesterase.

Particle Size - The influence of particle size on the overall absorption of medroxyprogesterone in man has been studied by Smith, Pulliam and Forist.[24] Utilizing an eight-hour urinary excretion of metabolite as an index of the absorption of drug, they found that 2.23 times as much medroxyprogesterone was absorbed from the micronized formulation as was obtained from the non-micronized drug formulation. While these data could also be interpreted to indicate only an increased rate of absorption with no change in the total amount of drug absorbed, the authors feel that because of the finite transit time of the drug through the gastrointestinal tract, and the previously reported excretion studies of Helmreich and Huseby,[25] these data are best interpreted to indicate an

enhanced total absorption of the drug.

Drug Interactions and Availability - The role of surfactants in modifying the availability and/or the absorbability of various barbiturates via the rectal route was the subject of a report by Fincher, Entrekin, and Hartman.[26] These workers, employing a petrolatum-paraffin base suppository, added various surfactants of known HLB values, and determined the effect of the incorporated barbiturate on the rate of respiration of the rabbit. The authors concluded that while the inclusion of a surfactant enhanced the rate of absorption of the barbiturates in some cases, it could also bind the drug, thus making it less available for the absorption process. The chemical type of drug and surfactant were deemed more important than either the apparent HLB of the system or the relative distribution coefficient.

Similarly, Levy, Miller, and Reuning[27] found that by adding various concentrations of polysorbate 80, a nonionic surfactant, to aqueous drug solutions, the absorbability of a barbiturate could be both increased or decreased. These authors employed goldfish as the test species and investigated the lethality times of low molecular weight alcohols and two barbiturates. The inclusion of the polysorbate 80 had no effect on the lethality times of the alcohols while it was concluded that low concentrations of the surfactant (below the critical micelle concentration) enhanced the absorption of sodium secobarbital, and higher concentrations decreased the rate of absorption of the barbiturate. In both this study and the work of Fincher et al,[26] the possibility of drug-surfactant interactions detrimental to the availability of the drug for absorption is a probability worthy of further study.

Singh et al[28] studied the interaction of various barbiturates with polyethylene glycol 4000 and found that phenobarbital formed a complex with the glycol. The solubility of the complexed phenobarbital was much lower than the intrinsic solubility of phenobarbital in the absence of polyethylene glycol 4000. Utilizing the everted sac technique to measure the rate of intestinal permeability, they found that the decreased solubility greatly decreased the rate of permeation. The other three barbiturates studied, pentobarbital, barbital, and barbituric acid, did not interact with the polyethylene glycol 4000 and the inclusion of this material did not decrease their rates of intestinal permeation.

Sorby and Liu[29] investigated the effect of adsorbents on the intestinal absorption of promazine in humans. The

results of this study point out the importance of knowing the reactivity of the drug with materials either contained in the dosage form or administered as concurrent therapy. They found that an antidiarrhea mixture containing attapulgite and pectin had a very strong affinity for promazine by in vitro adsorption techniques. When promazine was administered to humans along with the attapulgite-pectin mixture, the rate and extent of gastrointestinal absorption of promazine was decreased.

Influence of Dosage Form - The oral absorption of indoxole, an experimental non-steroidal anti-inflammatory compound, was studied by Wagner, Gerard, and Kaiser.[30] These workers employed four different dosage forms of indoxole in their studies, an emulsion, soft elastic capsules, an aqueous suspension, and a hard capsule. They found that solutions of the drug (the emulsion and the soft elastic capsule) gave superior absorption characteristics as measured by drug plasma levels than did the solid drug forms (the suspension and the hard capsule). It would appear that indoxole represents another case where dissolution rate and/or drug availability is the rate determining process in the over-all absorptive process.

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## Chapter 34. The Use of Substituent Constants in Drug Design

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The subject of structure-activity relations in medicinal chemistry is almost boundless. Few papers are now published without some discussion of this problem. The work considered in this review is limited to that concerned with mathematical correlations between structure and activity.

The use of physical-chemical reference systems to serve as scales with which one could discuss in quantitative terms the relation between biological activity and chemical structure excited great interest at the turn of the century. C. Richet, J. Traube, H. Führner and especially H. Meyer and E. Overton gave thrust to this movement. It was observed, almost entirely in aliphatic systems, that as one increased the chain length in a homologous series a regular increase in a standard biological response occurred. Linear relations between a standard response and the number of carbon atoms in the chain, water solubility, surface tension lowering ability, vapor pressure and oil-water partition coefficients were often found in many different tests. An example of this type of correlation using partition coefficients coming from the work of Overton is:

$$\log 1/C = 0.858 \log P + 0.837 \qquad n = 28 \qquad r = 0.978 \tag{1}$$

In eq 1, C represents the molar concentration of various alcohols, ketones and esters producing isonarcosis in tadpoles, P is the octanol-water partition coefficient, n is the number of data used in finding the constants via the method of least squares, and r is the correlation coefficient. The constants in eq 1 are slightly different from those published[1] and the correlation is a little better because of the use of improved P values.

An important advance was made by Ferguson[2,3] who showed that the following generalization holds.

$$C_i = kA_i^{1/n} \tag{2}$$

In eq 2, $C_i$ is the concentration of the $i^{th}$ member of a series producing an equivalent response, k and n are the constants, and $A_i$ is a physical-chemical distribution constant of the above mentioned kind. Ferguson pointed out that for nonspecific narcotics in states of equilibrium, biological activity is related to thermodynamic activity. Ferguson's approach was extended by Brink and Posternak[4] and it is now evident that there are many instances where equal degrees of narcosis are caused by molecules having equal thermodynamic activities. Other approaches to explaining the effect of narcotic activity in terms of physical-chemical parameters are those of McGowan[5-8] and Mullins[9]. McGowan using parachor and Mullins using Hildebrand's solubility parameter and molal volume, have

assembled evidence to support the view that the molecular volume of the narcotic is of critical importance in determining narcosis.

Pauling[10] and Miller[11] have suggested that anesthetic potency is related to the ability of anesthetics to form hydrates. Miller, Paton and Smith[12] and others[13] have criticized the Pauling-Miller view.

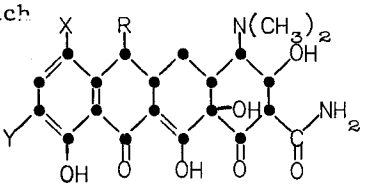
Agin, Hersh and Holtzman[14] have shown that eq 3 gives an excellent correlation for the relation between minimum blocking concentration (MBC) of 39 local anesthetics in frog sartorius muscle with polarizability ($\alpha$) and the ionization potential (I) of the drugs.

$$\log (MBC) \propto \alpha I \qquad (3)$$

Zahradnik[15], in a systematic study using mice and carefully controlled conditions, developed a set of constants for alkyl groups from the linear Hammett-like postulate that $\log (\tau_i/\tau_{Et}) = \alpha\beta$ where $\tau_i$ represents the molar concentration of the $i^{th}$ member of a set of aliphatic congeners producing a standard biological response and $\tau_{Et}$ is the concentration of the ethyl derivative. $\beta$ is a constant characteristic of the substituent (R of RX) and $\alpha$ is a constant characteristic of the system. The constant $\beta$ was determined for 25 R groups and $\alpha$ was evaluated for 39 different biological systems. While this single parameter approach works well for inhibitory studies of homologous groups of aliphatic compounds where highly specific electronic and steric effects are not critical, it has not been extended to more complex systems.

The above ideas have provided insight into nonspecific biological inhibitions; however, this knowledge has not been of great help in the design and modification of drugs of high specificity. Two approaches have been emerging in recent years to supplement the medicinal chemists' intuition on the effect of substituent variation on drug activity. In one aproach de novo substituent constants have been derived by simply finding the "best numbers" to go with given substituents on a particular drug acting in a standard test. The other approach has used substituent constants derived from model nonbiological systems. The first work on the former of these two tracks appears to be that of Bruice, Kharasch and Winzler[16] working with thyroxine derivatives. Their approach has been stated in more general terms by Free and Wilson[17]. The method can be illustrated with their example for a set of tetracycline (I) analogs. The relative biological activity for each derivative can be formulated as the linear combination of the contribution of each of the groups represented by X, Y and R:

$$\text{Biological activity} = \mu + a[X_i] + b[Y_i] + c[R_i] \qquad (I)$$



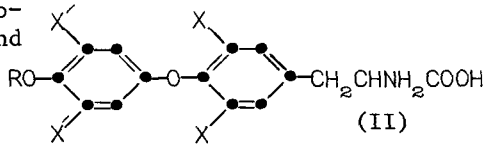

One can write a set of simultaneous equations, one for each compound tested, the solution of which gives the contribution to the activity for each $a[X_i]$, etc. Table I lists the results for the tetracyclines.

Table I

| R | | X | | Y | | |
|---|---|---|---|---|---|---|
| a[H] | 75 | b[Cl] | 84 | c[$NH_2$] | 123 | |
| a[$CH_3$] | -112 | b[Br] | -16 | c[$CH_3CONH$] | 18 | $\mu$ = 161 |
| | | b[$NO_2$] | -26 | c[$NO_2$] | -218 | |

Of the total of 18 possible derivatives resulting from different combinations of R, X and Y, 10 had been tested. Presumably, activities for the other eight can be calculated from the substituent constants in Table I. The method is of greatest value when a large group of derivatives and functions is involved. There are of course many objections that come to mind in considering the above technique. Modern chemistry has long recognized the great importance of steric and electronic effects of substituents on rate and equilibrium processes[18]. From the Meyer-Overton work and from more recent studies[19-25] the hydrophobic bonding power of functional groups is seen to be a very important substituent effect. These three effects are lumped into each of the constants under R, X and Y of Table I. Hence it would seem that in general, constants derived for one set of congeners will not be useful for another set causing a different biological response. Nevertheless, this empirical method of deriving constants for the relative effects of chemical groups on biological activity is bound to be more helpful in the long run than unguided intuition. Purcell[34] has been studying the Free-Wilson method.

Some information is already in hand to show that such empirical constants are related to more fundamental parameters. Cilento and Berenholc have shown[26] that there is a good linear correlation between f(X) obtained for[16] thyroxine analogs (II) and the negative logarithm of the lifetime of the phosphorescent state (equivalent to the triplet to singlet transition in naphthalene analogs) for the five functions where X = H, $CH_3$, Cl, Br, I. They conclude that the strong $T \leftrightarrow S$ transition in diiodotyrosine and thyroxine derivatives makes these compounds very efficient in the transfer of triplet-state energy. Using the substituent $\sigma$ for the electronic effect and $\pi$ for the hydrophobic effect[27] of substituents, it is seen from eq 4 that f(X′) derived by Bruice et al. is in fact related to these more fundamental constants.



(II)

$$f(X') = 0.308\pi - 0.564\sigma - 1.672 \qquad n = 7 \qquad r = 0.934 \qquad (4)$$

It has been shown[28] that $\pi$ and $\sigma$ can be used to rationalize the SAR of thyroxine analogs and Jorgensen[29] has tested the predictive value of the use of $\sigma$ and $\pi$ with a tert-butyl analog of thyroxine. It has also been shown[30] that Zahradnik's $\beta$ constants are linearly related to $\pi$.

Kopecký and Boček[31,32] have also been studying the utility of empirical substituent constants in an investigation of the toxicity of disubstituted benzenes. In their equation (5) they have used an interaction

term of the type discussed by Miller[33] for the linear combination of terms in the formulation of mathematical models.

$$\log \frac{[LD_{50}]HH}{[LD_{50}]XY} = bX + bY + eXeY \qquad (5)$$

In eq. 5, HH represents benzene and XY a disubstituted derivative. Quite good correlations were obtained using eq 5 for nonspecific toxicity. The constant bX is stated to be linearly related to f(X′) of Bruice et al.

Purcell[36], following this general approach, has studied amides which are inhibitors of cholinesterase. Purcell[35,36] and co-workers have also investigated a variety of physical chemical parameters to rationalize the SAR for these inhibitors.

After the rather intense work on the correlation of biological activity with partition coefficients of the early years of this century had reached a standstill, new hope for rationalizing the biological activity of organic compounds appeared in the work of Hammett[37]. The Hammett equation and other variations of it[18,38] have proved to be very successful in correlating chemical reactivity with the electronic or steric effects of substituents for the reactions of organic compounds in homogeneous solutions. Although a good many attempts have been made[39-47] to apply the Hammett equation to biochemical systems, the results have, with few exceptions, been disappointing. Such an exception is seen in the enzymatic hydrolysis of phenyl sulfates[39] from which eq 6 results[24].

$$\log 1/K_m = 0.930\sigma + 2.522 \qquad n = 10 \qquad r = 0.931 \qquad (6)$$

In eq 6, $K_m$ is the Michaelis constant. Good correlation with $\sigma$ was also obtained with $V_{max}$. The best linear relations with $\sigma$ have been found using more or less pure enzymes. The lack of success with $\sigma$ in biochemical systems has generally been attributed[48] to steric interactions of substituents with the enzyme or lipoprotein membranes. Recent work[21-25] would indicate that while steric interactions are extremely important, the concept of lock-and-key fit of enzyme and substrate has been over-emphasized at the expense of hydrophobic bonding. The importance to the medicinal chemist of the more flexible character of enzymes which is emerging from the work of Koshland[49] and others[50] has been analyzed by Belleau[51].

A most important new concept for the designer of drugs is that of the hydrophobic bond[19,20,52]. The view of Hansch and co-workers is that if a suitable parameter can be formulated for this, then by means of the well known constants[18] $\sigma$, $\sigma^+$, $\sigma^-$, $\sigma^*$, and $E_s$, many of the powerful tools of physical organic chemistry might be brought to bear on medicinal chemical problems. To this end octanol-water partition coefficients (P) have been studied as a reference standard[1,24,27]. The additive-constitutive nature of log P and $\pi$ ($\pi = \log P_X - \log P_H$ where $P_H$ refers to a parent molecule and $P_X$ to a derivative) means that from a relatively few values of log P, many others can be calculated[1,24,27,53,54]. The usefulness of these parameters for measuring binding of neutral molecules with proteins is illustrated in eq 7-9.

$$\log 1/C = 0.71\ \log P + 1.51 \qquad n = 17 \qquad r = 0.950 \qquad (7)$$

$$\log 1/C = 0.58\ \log P + 2.40 \qquad n = 4 \qquad r = 0.961 \qquad (8)$$

$$\log 1/C = 0.68\pi + 3.48 \qquad n = 19 \qquad r = 0.962 \qquad (9)$$

In eq 7-9, C is the molar concentration of compound necessary to produce a 1-to-1 complex with bovine hemoglobin[21] (eq 7) and bovine serum albumin (eq 8 and 9). Equation 7 correlates the binding of 17 miscellaneous compounds (e.g., phenols, anilines, naphthalene) and eq 8 correlates the binding of 4 barbiturates[24]. In eq 9, the comparative constant $\pi$ is used[55] to correlate the binding of phenols by serum albumin. The slopes of the three equations are surprisingly close, indicating that the binding of neutral molecules to two different proteins can be quantitatively defined using log P or $\pi$ as hydrophobic bonding constants. For these situations no highly specific steric or electronic parameters are necessary to rationalize the results.

Going to the next more complex situation, that of enzymic reactions, it can be shown that the linear combination of $\pi$ and $\sigma$ can account for the substituent effects in the hydrolysis of phenyl glucosides by emulsin[56]. Using regression analysis, eq 10-13 are derived to illustrate how one can factor substituent effects on a biochemical reaction.

Para Groups

$$\log K_e = 0.52\sigma + 2.03 \qquad n = 8 \qquad r = 0.753 \qquad (10)$$

$$\log K_e = 0.33\pi + 0.620 + 1.80 \qquad n = 8 \qquad r = 0.921 \qquad (11)$$

Meta Groups

$$\log K_e = 0.95\sigma + 1.63 \qquad n = 6 \qquad r = 0.949 \qquad (12)$$

$$\log K_e = 0.12\pi + 0.96 + 1.59 \qquad n = 6 \qquad r = 0.963 \qquad (13)$$

$K_e$ represents the equilibrium constant for the enzyme substrate complex. Comparison of eq 10 and 11 for the para derivatives indicates that complex formation depends on both electronic and hydrophobic factors. The correlation coefficient is much better and statistically quite significant for eq 11. The positive signs of the coefficients for $\pi$ and $\sigma$ indicate that lipophilic and electron-attracting groups promote complex formation. Comparing eq 12 and 13, one finds that for meta isomers hydrophobic bonding is apparently not possible. No significant improvement in correlation results on the introduction of the $\pi$ term. A similar set of equations was derived[56] to show the substituent effect on the hydrolysis rate constant $k_3$. Equally good correlations were obtained except in this step; as one might expect, the coefficient with $\pi$ has a negative sign. This indicates that hydrophobic bonding slows down desorption of the cleaved products. Since good correlations were obtained in eq 11 and 13 without the use of steric constants, it is assumed that these are unimportant, at least for functions as large as those studied.

The meaning of the words steric and electronic in these discussions is somewhat ambiguous. For example, the linear relations between parachor and toxicity found by McGowan or the use of the solubility parameter $\delta$ by Mullins could be viewed as indicating a direct relation between the size of the substituent and its ability to produce a given biological response. The affinity of an apolar group for a lipid phase will also be a function

of its size and, to a lesser extent, its shape. Thus it is not always easy, even in the abstract, to separate the binding role of hydrophobic bonding by an apolar function from its role of distorting a hydrophobic region by its size. We are attempting to use hydrophobic bonding as defined by partition coefficients as simple holding of the drug to the active site, realizing that often this role cannot be separated from the conformational change the apolar group will, in the binding process, produce in an enzyme or membrane. Steric effects, then, are those due to size or arrangement in space of substituents which cannot be accounted for by the hydrophobic constants log P and $\pi$. These will be both intra- and intermolecular in nature. In the same way, electronic effects will overlap with hydrophobic bonding since the position of equilibrium in the distribution of a drug between phases will be a function of its electronic structure. Indeed, it has been shown[27] that $\pi$ varies with $\sigma$. However, this variance is not great if the substituents are separated by one or more atoms. The term electronic effects means highly specific effects in general not associated with the partitioning process. These would be effects involved in a chemical reaction or charge transfer process where a change in electron density too small to make a significant difference in log P could cause a large change in a rate or equilibrium constant. Using these somewhat arbitrary divisions of substituent effects and regression analysis, a start can be made in separating the effects of groups of atoms on the biological activity of a set of congeners. Hansch and co-workers have not, for example, attempted to factor out hydrogen bonding. In general they have worked with systems where this could be accounted for in terms of $\sigma$ and $\pi$. Purcell et al. have suggested a way of dealing specifically with this term[36].

In addition to many early examples[15], biological response has been found to be quantitatively linearly dependent on log P or $\pi$ in the inhibition of the Hill reaction[57,58] activity of penicillins[59], toxicity of benzoic acids to mosquito larvae[28], phenol coefficients[28], cholinesterase inhibitors[35], and catechol-amine activity[60].

Hemker[61] was one of the first to attempt the quantitative correlation of biochemical response using both partition coefficients and ionization constants to account for the uncoupling action of phenols. The linear combination of $\pi$ and $\sigma$ has been found to hold for several enzymic reactions[22,56] as well as the binding of phenols by protein[55], the toxicity of phenols[62], the uncoupling action of phenols[62], and the relative sweetness of nitroanilines[23]. An interesting application is that of McMahon[63]. Equation 14 was formulated for the enzymic reduction of aromatic ketones to alcohols.

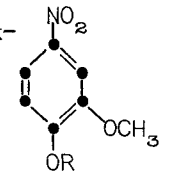
$$\log V_{max} = 0.334\pi + 1.239\sigma + 0.824 \qquad n = 10 \qquad r = 0.89 \tag{14}$$

Of course the most interesting and difficult drug studies are those in which whole organisms are involved. It has been postulated[64] that in general, for these studies one would expect a parabolic relation between log 1/C and log P. This has led to the development[28,65] of eq 15.

$$\log 1/C = -k(\log P)^2 + k' \log P + \rho\sigma + k'' \tag{15}$$

In the development of this model a probabilistic view is taken for movement of drug from the exobiophase or point of injection to the sites of action. It is assumed that as $P \to 0$ drugs become more and more isolated in the water phase and eventually become unable to cross lipid barriers. As $P \to \infty$ the reverse is true. Somewhere between $P=0$ and $P=\infty$ there will be an ideal value ($P_o$) for a given set of congeners in a given biological system such that those members having this value will find the sites of action via a random walk process in the minimum time. This assumes steric and electronic ($pK_a$, etc.) factors are constant. In other words, a greater number of molecules of drug with $P_o$ would reach the sites of action in the test interval than drugs having other P values. In effect, one expects a change in the mechanism of movement in a set of drugs having a sufficient spread in P values. The movements of the lower members of the series will be mostly determined by interactions with water, while those of the higher members will be determined by hydrophobic interactions with lipids. Such parabolic relations may even be found in closed systems of the type used in narcosis studies. Ferguson[66] has stated that probably in most experiments on narcosis a complete equilibrium is never reached in the period of exposure and the results are time dependent. Results will be even more time dependent in open systems such as whole animals where elimination and biotransformation are continuous processes. Such results can even be expected in tissue experiments or work with partially purified enzymes. Since activity is usually expressed as log 1/C, the most active compounds tested are often in very low concentration, sometimes less than $10^{-6}$ M. While log 1/C may be linear with respect to log P at higher concentrations eq 6-8 indicate that highly lipophilic molecules will be very tightly bound to proteins so that true equilibrium is not reached in test time. The lower the test concentrations become, the more likely the departure from linearity through localization of molecules in particularly lipophilic material.

Of course there are reasons other than binding by lipids or proteins which might cause a departure from linearity in the relationship of log 1/C and log P or $\pi$. Metabolic or elimination reactions not significant at low values of log P could, with increasing log P, become very important. A good example of this is in the metabolism of alkylaryl ether in rabbits [67]. In (III) when R is ethyl or methyl, dealkylation is the main reaction. When R is propyl or butyl, $\omega$-1 hydroxylation becomes more important. Since the rate of metabolism of drugs may be linearly related[68] to log P, this may be an important contributing factor for the parabolic dependence of activity on lipophilic character. Another unknown is the lipophilic space at the site of action available for the hydrophobic moiety of the drug. If this is quite limited, then a point is soon reached where log 1/C and log P are no longer linearly associated. The probabilistic nature of the log P terms in eq 15 does a good deal to insure a reasonable fit of a set of data as long as individual congeners in the set do not depart radically from the behavior of those having similar lipophilic character. In principle, loss through metabolism or elimination is not different than loss through binding if these losses depend only on log P. Good results have

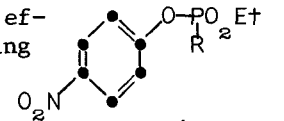


(III)

been obtained with eq 15 for plant-growth regulators[65], chloromycetin analogs[65] (Garrett[69] and colleagues have found a more linear dependence of chloromycetin activity under different test conditions), thyroxine analogs[28], phenol coefficients[28], carcinogenicity of aromatic compounds[28], and the localization of benzeneboronic acids in brain and tumor tissue[70].

A constant which may prove to be very useful in drug design is log $P_o$ (or $\pi_o$). This figure can be found by taking the partial derivative of eq 15 and setting this equal to zero. Once this has been established for a set of drugs, it becomes a useful bench mark from which to start the design of a completely new drug to act on the same sites. For example, it was found that log $P_o$ for phenoxyacetic acids acting as plant-growth regulators is 2.03. Log $P_o$ for phenylacetic acids acting in the same system is 2.47. If one wished to design a new acid to act in this system, one of the features which one would design into the first test molecule would be log P of about 2.2. By means of the additive-constitutive character of log P one could design such compounds on paper without carrying out extensive partition coefficient studies.

While suitable techniques to handle steric effects between substrate and the material comprising the sites of action are at present out of reach, intramolecular steric interactions can be handled quantitatively, at least in some instances using Taft's $E_s$ parameter[22,56]. Equations 16 and 17, formulated from the work of Metcalf and Fukuto correlate[22] substituent effects of R in (IV) on the inhibition of cholinesterase by alkylphosphonic acid esters.




(IV)

$$\log K = 3.74\ E_s + 7.54 \qquad n = 13 \quad r = 0.901 \qquad (16)$$

$$\log K = -1.68\sigma^* + 0.15\pi + 4.05\ E_s + 7.21 \qquad n = 13 \quad r = 0.907 \qquad (17)$$

Comparison of eq 16 and 17 indicates that neither electronic nor hydrophobic factors play an important part in the inhibition. Thus it appears that the phosphonates interact with the enzyme in such a way that they cannot come in contact with a hydrophobic region of the protein. The range of electronic forces covered by the R groups was small so that $\sigma^*$ may be more important than eq 17 would indicate. Some overlap between $E_s$ and $\sigma^*$ constants tends to obscure the role of $\sigma^*$. The fact that $E_s$, a constant derived from hydrolysis studies under homogeneous conditions, should apply to heterogeneous catalysis is surprising and indicates much more use for this parameter than one would have had reason to expect.

Another method for obtaining biochemical substituent constants is available in the various forms of chromatography. Considerable effort[71-73] has been made to gather $R_M$ and $\Delta R_M$ values for the effect of substituents on $R_F$.

$$R_M = \log(1-R_F)/R_F \qquad \Delta R_M = R_{M_X} - R_{M_H} \qquad (18$$

In eq 18, $R_{M_H}$ is calculated from the $R_F$ value of a parent compound and $R_M$ from that of a derivative having substituent X. $\Delta RM$ is a constant analogous to $\pi$ and in fact a close correlation exists between the two as is

shown[1] in eq 19.

$$\pi = -1.103\Delta R_M + 0.647 \qquad n = 12 \qquad r = 0.970 \tag{19}$$

In measuring the $\Delta R_M$ values in eq 19, Green and Marcinkiewicz[71] used trigol and diisopropyl ether in reversed-phased, tankless flat-bed chromatography. Boyce and Milborrow[74] have shown that $R_M$ values correlate the molluscicidal activity of a series of N-n-alkyltritylamines assuming a parabolic reaction exists between log $LD_{50}$ and $R_M$. Thus $R_M$ or $\Delta R_M$ values can be used in eq 15 in place of log P or $\pi$.

Considerable effort[35,75-80] has been made to understand biochemical and pharmacological problems using quantum mechanical calculations of electron densities with molecules. While this has been useful in explaining how a particular bond is broken or made[75], linear relations between calculated electron densities and biological activity are notably lacking. An exception coming from the work of Fukui is illustrated in eq 20 and 21 which correlate the nicotine-like activity of meta derivatives of $C_6H_5OCH_2CH_2\overset{+}{N}(CH_3)_3$ with the superdelocalizability ($S_o^{(N)}$) of electrons at the ortho position and the frontier electron density ($f_{oxy}^{(E)}$) on the ether oxygen.

$$\log A = 13.742\ S_o^{(N)} - 10.465 \qquad n = 6 \qquad r = 0.994 \tag{20}$$

$$\log A = 30.392\ f_{oxy}^{(E)} - 20.924 \qquad n = 6 \qquad r = 0.949 \tag{21}$$

The combination of such electronic densities with a hydrophobic bonding constant does yield good results[28,56] as illustrated[56] by eq 22 and 23.

$$\log A_X = 22.91\varepsilon - 42.49 \qquad n = 6 \qquad r = 0.685 \tag{22}$$

$$\log A_X = 0.29\pi + 18.16\varepsilon - 33.82 \qquad n = 6 \qquad r = 0.995 \tag{23}$$

In the above equations $A_X$, from the work of Jacobson, represents the relative rates of acylation of aromatic amines having substituents X by means of pigeon liver acetyl transferase. $\varepsilon$ represents the calculated electron densities on the nitrogen atom made by Perault and Pullman. Equation 22 accounts for only 47% of the variance in the data while eq 23 accounts for 99%. Thus it appears that quantum mechanically obtained electron densities can be extremely useful to the medicinal chemist. In this way the relative electron density on each atom in the molecule can be found and through regression analysis the relative importance of this density at one or more points in the molecule can be evaluated. This approach offers, in principle, a great advantage over the use of $\sigma$.

What are the guidelines that substituent constant analysis has to offer the designer of drugs? The great success of this method in homogeneous organic reactions[18] and the more modest achievements of this method with heterogeneous biochemical reactions would seem to validate the use of eq 24 and 25 as a reasonable working hypothesis.

$$\Delta F^\circ_{BR} = \Delta F^\circ_{L/H} + \Delta F^\circ_{elect} + \Delta F^\circ_{steric} \propto \log k_{BR} \tag{24}$$

In eq 24 we are assuming[57] that the ultimately measured biological response (toxicity, resistance to a metabolic process, $ED_{50}$, elimination,

etc.) is governed by one rate-limiting process for which $k_{BR}$ is a rate or equilibrium constant. In eq 24, $\Delta F^{\circ}_{L/H}$ represents that portion of the free energy change which can be attributed to hydrophobic bonding, $\Delta F^{\circ}_{elect}$ represents an electronic component, and $\Delta F^{\circ}_{steric}$ represents highly specific spatial demands of reactants and products on the free energy change. Of course, were sufficient data available, one might profitably factor each of the terms in eq 24 and 25 into several to represent suspected critical parts of a molecule. Substituent effects on log $k_{BR}$ of eq 24 are represented in eq 25.

$$\delta_X F^{\circ}_{BR} = \delta_X F^{\circ}_{L/H} + \delta_X F^{\circ}_{elect} + \delta_X F^{\circ}_{steric} \propto \delta_X \log k_{BR} \qquad (25)$$

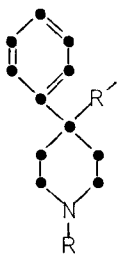
Equations 24 and 25 were formulated for the case where the drug is at the site of action or the situation where true equilibrium with the exobiophase is established as envisaged by Ferguson.

From the present limited work it would appear that $\delta_X F^{\circ}_{L/H}$ might be represented by log P, $\pi$, $R_M$, $\Delta R_M$, $\beta$ (Zahradnik[15]) and, under certain conditions, parachor. The $\delta_X F^{\circ}_{elect}$ term may be represented using the various forms[18] of $\sigma$, quantum mechanically calculated electron densities or chemical shifts[81] obtained via NMR. It should also be possible to formulate suitable constants from IR and UV spectra. Attempts to use polarographically-obtained constants do not seem to be as useful as one might expect[82,83].

The case where substituent changes result in large differences in ionization is one of great importance since so many drugs are either weak acids or weak bases. The degree of ionization has long been recognized as playing a part in drug activity[84]. In a careful analysis, Fujita[62] has shown how substituent effects on ionization should be separated from other electronic effects of substituents.

It does not seem possible to make any general observations about suitable ways of representing $\delta_X F^{\circ}_{steric}$ for the interactions between a drug and its receptors. By considering sets of congeners in which gross steric changes are avoided (e.g., considering D isomers separately from L isomers, etc.) useful correlations can be made. In fact, where the two-parameter equation can be shown to hold over a reasonable range of substituents, one can make deductions about $\delta_X F^{\circ}_{steric}$ by continuing to increase the size of X until the two-parameter equation fails. In this way one can, to a limited extent, map the free space around a receptor site.

Portoghese[85-87], in an extension of the approach of Zahradnik, has shown that linear relationships between different congeneric sets of analgesics can be used to make more firm decisions about whether the sets are acting in the same three-dimensional way on the same receptors. The three sets of congeners in which R of V was held constant (i.e., 1. R′ = $CO_2Et$, 2. R′ = OCOEt and 3. R′ = $OCOCH_3$) were varied by changes in R. Least square fits of sets 1 vs. 2, 1 vs. 3, and 3 vs. 2 gave



(V)

slopes close to 1 with good correlation coefficients. Such tests can be used to help establish the fact that, for example, R in each series is in the same physicochemical environment on the receptors. By exploring a large enough number of sets of systematically varied congeners one could obtain considerable information about the geometry of the receptor sites.

The fact that simply changing the length of R can lead to a complete change in the mechanism of action (e.g., agonist to antagonist) has been thoroughly documented by Ariëns[88]. A careful case study in which the enthalpic and entropic roles of the substituents in transition from agonist to antagonist have been considered has been made by Belleau, Tani and Lie[89]. Miller and Hansch[25] have presented evidence to show that when two hydrophobic areas are present in a drug and only a limited space for hydrophobic bonding exists in an enzyme, the more hydrophobic of the two groups may determine the configuration of binding.

Thus steric interactions of receptor site and substrate are extremely difficult to evaluate. The conformational perturbations of drug on receptor and receptor on drug will tax to the limit our ability to untangle the mechanism of drug action for many years to come. However, the use of substituent constants and large computers for regression analysis offers us new hope denied workers a few years ago.

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# SUBJECT INDEX