LIST OF CONTRIBUTORS

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Preface

While excellent reviews in depth covering selected fields of medicinal chemistry are available, no publication outlines the current developments and trends in the whole area. The present volume is the first of a planned annual publication in which more than thirty authors present a critical summary of new and significant contributions appearing in the literature of the past year concerning various fields of medicinal chemistry. Its main purpose is to enable readers to "catch up" in fields of their peripheral interests and, perhaps, to find some different views in fields of their major interests.

The breadth of the subject and limitations of space presented to the authors a very difficult task of selection of material and condensation of discussion. Recent reviews and leading references are cited to guide readers to further information if desired. Topics were chosen to achieve reasonable coverage, but a few chapters initially listed had to be postponed to future volumes. Organization of the material is primarily by pharmacological action, although some chapter titles represent concepts, chemical classes, methods, etc. The manner of presentation varies, as is to be expected from the various disciplines of the authors, the nature of the subjects and the lack of a previous volume to serve as a model.

I know of no adequate way to express my thanks for the cheerful, enthusiastic and dedicated efforts of all who were involved in this undertaking. Especially to be mentioned are the section editors and the chapter authors, but secretaries, proof-readers, assistants and all who supplied moral support and encouragement should not be forgotten.

Comments, criticisms, suggestions and, hopefully, praise will be welcomed by authors and editors.

Fort Washington, Pa.
June, 1966

Cornelius K. Cain

Section I - CNS Agents
Editor: John H. Biel, Aldrich Chemical Co., Milwaukee, Wisconsin

Chapter 1. Antipsychotic and Anti-anxiety Agents

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

Most basic biological work concerned with CNS agents that was reported in 1965 falls into the biochemical area. The belief that the antipsychotic drugs function through some interference with adrenergic processes in the brain is widely accepted. Reserpine, for example, is known to deplete the brain of its stores of catecholamines whereas chlorpromazine has a central adrenolytic action. Although the antipsychotic agents can be shown to interfere with the brain amines, the causal relationship between the changes and the behavioral effects is less clear. The mode of action of the anti-anxiety drugs remains unknown.

A symposium on catecholamines held in Milan in 1965 and recently published[1] does much to clarify present views of their role in the central nervous system. Other reviews on the biochemical effects of drugs acting on the central nervous system and on the pharmacology of the central nervous system were prepared by Decsi[2] and by Bradley.[3] More detailed reviews on serotonin[4] and γ-aminobutyric acid[5] appeared. These papers provide excellent background for any fundamental consideration of the CNS drugs.

If the brain amines are conceded to play a crucial part in the functioning of the central nervous system, some disorder in the supply, action or disposal of these agents might be responsible for abnormal behavior. Gellhorn[6], for example, has hypothesized that a disturbance in the noradrenaline/adrenaline ratio forms a neurophysiological basis for fear and anxiety. The suggestion that toxic substances in the brain might be responsible for schizophrenia is an old one and the search for a faulty metabolic process in schizophrenics has remained a popular pursuit. Bourdillon[7] has recently reëmphasized the finding of a "pink spot," probably 3,4-dimethoxyphenethylamine, in the urine of schizophrenics. Work by Ernst[8] supports the possibility of an abnormal metabolism of dopamine to 3,4-dimethoxyphenethylamine in schizophrenia. He studied the structure-activity relationships of a group of methoxylated phenethylamines in the production of catatonia in cats. He found the presence of a p-methoxy group and the absence of m-hydroxy group to be required with the duration of action determined by the number of methoxy groups in the molecule. Prior treatment with iproniazid eliminated the requirement for the absence of a m-hydroxy group. The blocking of the amino-oxidase resulted in m-methylation by O-methyltransferase. A behavioral study[9] of this compound in the dog and cat also indicates some relationship to schizophrenia.

Woolley and Gommi[10] have detected in the blood of schizophrenics a substance that is synergistic with serotonin in its action on the rat uterus and are studying the possibility of a causal relationship to the mental disorder.

An excellent review[11] on biochemistry and mental function has been published by Kety who has also republished his 1959 review[12] criticizing some

of the methodology involved in finding the "needle-in-the-haystack" that might explain the causes of schizophrenia. Six rejoinders from other scientists are included.

Most of the compounds cited below were tested by two general methods: 1) the study of animal behavior following drug treatment in experimental models designed to mimic a clinical situation (conflict, avoidance, etc.) and 2) the measurement of classical pharmacological reactions caused or modified by the agent under test (anticonvulsant, antiemetic, etc.). These methods have been collected in a recent Hahnemann symposium volume.[13] The testing of the antipsychotic compounds has been reviewed by Janssen, et al.,[14] who rely strongly upon the production of catalepsy and the antagonism of the emetic effect of apomorphine for predicting the clinical response. In fresh work on test methods, Marriott and Spencer[15] have observed that the anti-anxiety agents increase exploratory behavior in inexperienced rats whereas the antipsychotic compounds reduce such activity. The use of a treadmill in approach and avoidance studies of anti-anxiety compounds has been described by Gluckman.[16] A criticism of some of the behavioral tests used for evaluating anxiety in animals has been made by Ray.[17] The electronencephalographic effects of the psychotropic agents have been reviewed.[18]

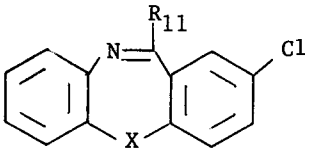
Methods of clinical study of psychotropic drugs have also been discussed and criticized.[19,20]

Phenothiazines and Analogs - Chemical work directed toward modification of the phenothiazine drugs is presently characterized by the preparation of novel tricyclic systems to which the common basic side chains can be attached. Variation of the central ring is most frequent but the peripheral rings are attracting some attention.

A group of compounds having a seven-membered central ring has been described by Stille, et al.[21,22] The synthesis of the intermediate lactams was accomplished by ring closure of the appropriate isocyanates.[23] The most active of these compounds is the oxazepine (I) which is approximately equipotent with haloperidol in cataleptic and antiapomorphine effects. In a more extensive study of the thiazepine analog (II, clothiapine) an intense antiserotonin effect was measured in the paw-edema test, thus differentiating it from chlorpromazine. It is noteworthy that opening of the piperazine ring as in compound III practically eliminates activity.

The Czech group led by Protiva prepared a host of compounds containing seven-membered rings. Two of these having good central depressant effects are IV (octoclothepine) and V.[24] Compound IV which has antiserotonin and antihistamine activities as well, is about three times as strong in its central depressant effects as its unchlorinated analog. The compounds were prepared conventionally from the corresponding dibenzothiepinone which, in turn, resulted from ring closure of the requisite o-phenylthiophenylacetic acid.[25]

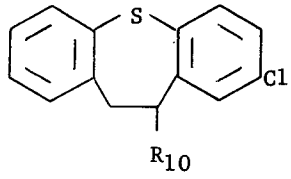
Reports have been made on several thioxanthene derivatives: VI (thiothixene),[26,27] VII (SKF 10812),[28,29] and VIII (N-7009).[30] Each was indicated to be effective in the treatment of psychotic states. Thiothixene was said to cause only a low incidence of extrapyramidal symptoms.



I: X=O; R_{11}= -N NCH$_3$

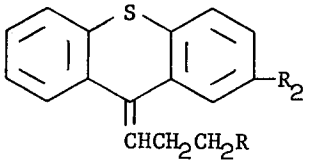
II: X=S; R_{11}= "

III: X=S; R_{11} = -N(CH$_3$)CH$_2$CH$_2$N(CH$_3$)$_2$



IV: R_{10}= -N NCH$_3$

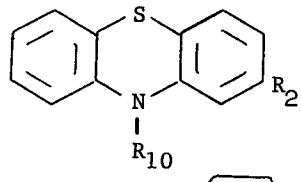
V: R_{10}= -OCH$_2$CH$_2$N(CH$_3$)$_2$



VI: R_2= -SO$_2$N(CH$_3$)$_2$; R= -N NCH$_3$

VII: R_2= -CF$_3$; R= -N(CH$_3$)$_2$

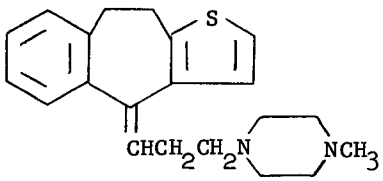
VIII: R_2= -CF$_3$; R= -N NCH$_2$CH$_2$OH



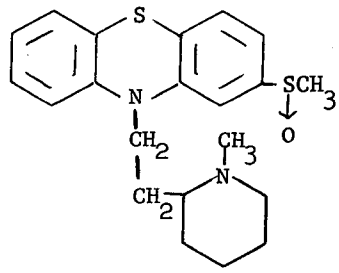
IX: R_2= -CN; R_{10}= -(CH$_2$)$_3$N OH

X: R_2=H; R_{10}= -CH$_2$CHCH$_2$N NCH$_2$CH$_2$ O CH$_2$ CH$_2$OH (CH$_3$)

XI: R_2= -CF$_3$; R_{10}= -(CH$_2$)$_3$N NCH$_2$CH$_2$OH



XII



XIII

Propericiazine (IX)[31] has been tried in psychopathic patients having severe behavioral disorders with fair success and dixyrazine (X)[32] has been found effective in anxiety associated with autonomic disturbances. Compound XI,[33] available in Europe, is a homolog of fluphenazine. Although it produces catalepsy it does not have antiemetic properties.

Neuroleptic activity has been reported for XII[34] which has a novel ring system.

A metabolite of thioridazine has been identified as XIII and shown to have a much lower neuroleptic potency than its parent.[35] Since there was some suggestion of activating properties (suppression of the tetrabenazine syndrome in rabbits, potentiation of serotonin fever and central anticholinergic activity) it was tested clinically as antidepressant agent with negative results.[36] However the activity in schizophrenia seems to be good.

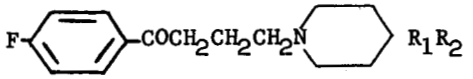
In the continuing study of the metabolism of chlorpromazine, 2-chlorophenothiazine and 2-chlorophenothiazine 5-oxide have now been identified in human urine.[37]

Butyrophenones - None of the fluorobutyrophenone compounds is at present in use in the United States although they are extensively used in Europe. The recent review by Haase and Janssen is a useful summary of these compounds.[38] An extension from their use in psychiatry to use in surgical procedures for producing analgesia is underway.[39,40] The appearance of several clinical studies[41] on the psychiatric use of trifluperidol indicates the prospect of its future availability for treatment of schizophrenia. There are some reports of its especial value in paranoid schizophrenia.[42]

Pharmacological and clinical data on compound XIV[43] show it to resemble haloperidol except for the addition of an antireserpine effect. A homolog of XIV (XV)[44] has also been studied but was found to have troublesome side-effects. Both of these products are somewhat related to XVI[45] which, although having an effective antipsychotic action, caused cataracts and disturbances of cholesterol balance.

A group of fluorobutyrophenones derived from 4-aminopiperidines has been described.[46] The treatment of 1-benzyl-4-t-aminopiperidine-4-nitriles with Grignard reagents effected displacement of the cyano group whereas organolithium reagents reacted with the cyano function to afford ketones. Subsequent debenzylation and alkylation gave the corresponding fluorobutyrophenones. These compounds are less active than haloperidol in apomorphine antagonism but are more potent in countering morphine-induced mania in cats. The most potent of these preparations is XVII.

A strong neuroleptic of long duration related to the butyrophenones has recently been reported.[47] Compound XVIII antagonizes the effect of apomorphine for as long as 100 hours.

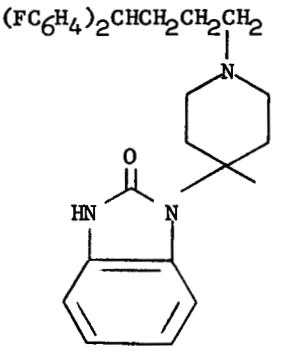


XIV: R_1= -CH_3; R_2= -H

XV: R_1=R_2= -CH_3

XVI: R_1R_2= -$CH_2CH_2CH_2CH_2CH_2$-

XVII: R_1= -COC_2H_5; R_2= -N



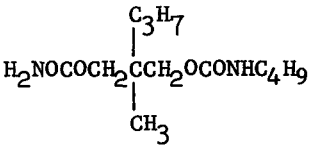
XVIII

Carbamates - A new carbamate, tybamate (XIX)[48] closely resembling meprobamate in structure and in activity was marketed during 1965. Its potency is comparable to meprobamate in many tests, yet it has only one-third the potency of meprobamate in the antipentylenetetrazole test. However, in contrast to meprobamate, it antagonises the effect of LSD on the electroencephalogram and has an antiserotonin action. Convulsions are not seen in dogs receiving tybamate upon abrupt withdrawal of the drug, whereas convulsions do result upon withdrawal of meprobamate.[49] In the dog tybamate is metabolized by hydroxylation and/or N-dealkylation.[50] Hydroxytybamate is the principal metabolite found in the urine.

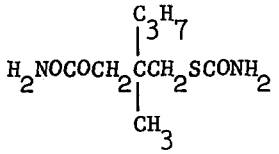
Several clinical studies have appeared demonstrating effectiveness against anxiety.[51,52] One report[53] suggested mild stimulant properties, but in a study with alcoholics no separation from placebo could be made.[54]

A series of meprobamate analogs was prepared containing silicon in place of the quaternary carbon atom.[55] Each silicon compound is approximately equivalent to its carbon analog in the rotarod test and in acute toxicity. A monothiol analog (XX) of meprobamate is also comparable to meprobamate in potency and toxicity.[56]

A chemical review of the carbamates has been written by Adams and Baron[57] with some attention to biological activity.



XIX



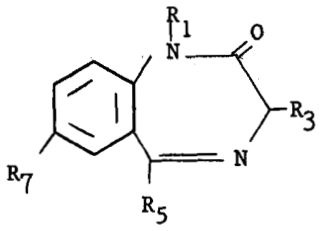
XX

Benzodiazepines - The introduction of oxazepam in 1965 brought to three the number of 1,4-benzodiazepines commercially available in the United States. The animal studies of oxazepam (XXI)[16,58,59] particularly the anticonvulsant and conflict tests, suggested its use as an anti-anxiety agent and clinical studies[60,61] indicated its efficacy for this purpose. Its potency appears to lie between chlordiazepoxide and diazepam. The compound has also been examined as a water-soluble hemisuccinate ester, sodium salt.[62]

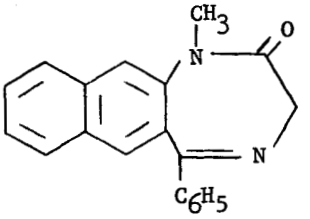
In the form of its glucuronide, oxazepam is the principal excretion product in the dog and man of diazepam (XXII) which is also hydroxylated to some extent without N-demethylation.[63,64] In the circulating serum, the most prominent metabolite of diazepam is the unhydroxylated compound XXIII. There is an indication of the presence of further phenolic metabolites whose precise structures are unknown. The metabolism of chlordiazepoxide in the dog and man results principally in the production of 7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide, but in the rat basic compounds as yet unidentified are produced.[65] Some opening of the lactam ring was observed, but no alteration of the aromatic rings or reduction of the N-oxide function was detected.

Nitrazepam (XXIV)[66,67] has been introduced in Europe and is being promoted as a hypnotic but its activity profile suggests it would be effective against anxiety. A clinical report on a related compound (XXV)[68] indicates a resemblance to diazepam.

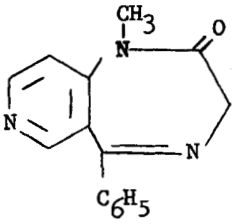
Some additions to the list of benzodiazepinones having functional substituents have been made. French workers succeeded in preparing 3-carboalkoxybenzodiazepinones, e.g. XXVI,[69] by transimidation of the appropriate 2-aminobenzophenone imines with α-aminomalonic esters followed by cyclization. The benzodiazepine esters so obtained were converted to amides and also hydrolyzed to salts of the corresponding carboxylic acids. The ease of decarboxylation upon acidification of these salts suggests that the decarboxylation products may be responsible for the high biological activity. Although the esters and amides have typical benzodiazepine profiles they are not so potent as the carboxylic acid salts which compare favorably with diazepam.



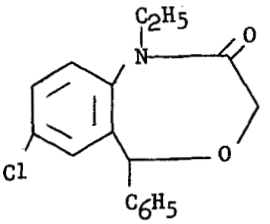
	R_1	R_3	R_5	R_7
XXI:	H	OH	C_6H_5	Cl
XXII:	CH_3	H	C_6H_5	Cl
XXIII:	H	H	C_6H_5	Cl
XXIV:	H	H	C_6H_5	NO_2
XXV:	CH_3	H	o-FC_6H_4	Cl
XXVI:	H	CO_2Et	C_6H_5	Cl
XXVII:	$CH_2CH_2N(C_6H_5)_2$	H	o-FC_6H_4	Cl



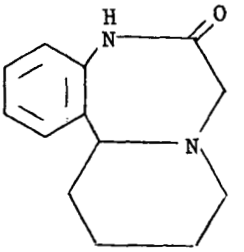
XXVIII



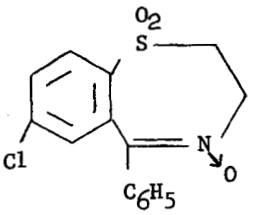
XXIX



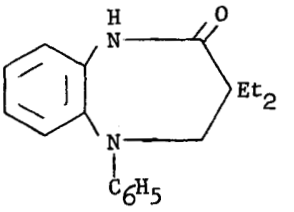
XXX



XXXI



XXXII



XXXIII

3-Acetamidobenzodiazepines were prepared by cyclization of 2-(2-acetamido-2-aminoacetamido)benzophenones.[70] A novel elimination of acetic acid from the appropriate 2-(N-acetoxyacetamido)acetamidobenzophenone afforded the intermediate. It was possible to hydrolyze the acetamidobenzodiazepines to the corresponding 3-amino compounds and convert these products into 3-hydroxy compounds by treatment with nitrous acid. The amino and acetamido compounds are merely reported to be active.[71]

Stempel, et al.,[72] achieved a direct synthesis of a 3-chlorobenzodiazepinone 4-oxide by base treatment of 6-chloro-2-dichloromethyl-4-phenylquinazoline 3-oxide. The course of this ring enlargement, which was responsible for the original discovery of chlordiazepoxide, was clarified by the isolation of an intermediate, 2-dichloroacetamido-5-chlorobenzophenone oxime (anti), which slowly cyclized. Cyclization of the monochloroacetyl analog is too fast to permit its isolation. The 3-chloro substituent of the benzodiazepine product reacted conventionally with nucleophiles following removal of the N-oxide function.

A number of benzodiazepinones with functional substituents in the 1-position has been published along with test data.[73] The general structure-activity requirements already described for the benzodiazepines obtain for the 1-aminoalkyl types. The importance of an o-fluoro substituent on the 5-phenyl ring in increasing the potency of the compounds is clear. Compound XXVII is one of the most potent of the group, but it is slightly less potent than diazepam. The activity of the related 1-aminobenzodiazepines prepared by use of chloramine was not given.[74]

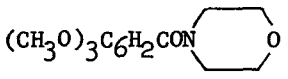
Compounds containing a fused naphthalene ring (XXVIII)[75] and a fused pyridine ring (XXIX)[76] have been disclosed. The former types are inactive and the latter are less active than diazepam. Further examples of compounds related to active benzodiazepines, but for which test data are missing, include XXX,[77] XXXI,[78] XXXII[79] and XXXIII.[80]

Miscellaneous Compounds - Investigations on several compounds that do not fall into the above groups were reported. The pharmacology of trimetozine (XXXIV)[81] indicates it to be a tranquillizer without hypnotic or anticonvulsant properties. Compound XXXV[82] has sedative properties approximating chlorpromazine but does not produce catalepsy and ataxia. A disruptive effect on conditioned behavior at very low dosage is seen with the adrenolytic compound XXXVI.[83] Compound XXXVII[84] is less effective than meprobamate against anxiety and it has been shown that poor absorption is not the explanation.[85] The tranquillizing potency of XXXVIII[86] is approximately equivalent to meprobamate.

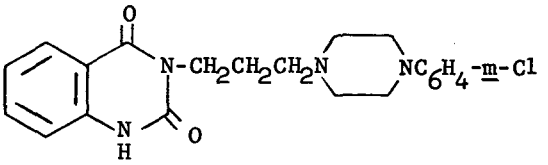
Taborsky, et al.,[87] have studied the effect of 1-methylation on a group of psychoactive indoles. In general, the effects on behavior of the methylated and unmethylated pairs are similar.

Compound XXXIX,[88] which resembles tetrabenazine in structure, has both stimulant and depressant properties. It blocks conditioned avoidance in rats but the required dose is at least five times that of chlorpromazine. Replacement of the p-chlorophenyl groups by an alkyl or aralkyl group leads to inactive products. A good clinical response in schizophrenics has been noted. A group of 17-haloyohimbanes[89] was examined by observation of the effects on

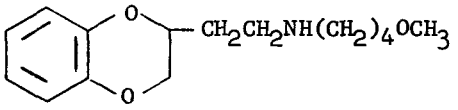
the behavior of untamed rhesus monkeys. Although 17-α-bromoyohimbane is approximately as potent as reserpine in altering behavior it has undesirable cardiovascular properties.



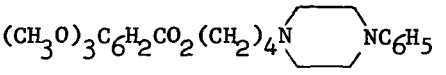
XXXIV



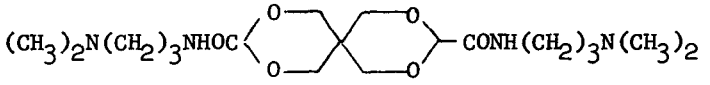
XXXV



XXXVI



XXXVII



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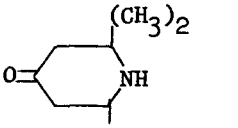

XXXIX

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Chapter 2. Antidepressants, Stimulants, Hallucinogens

J. H. Biel, Aldrich Chemical Company, Milwaukee, Wis.

I. The Antidepressants

A. Introduction

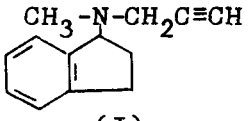
There were no spectacular break-throughs in the treatment of mental depression during 1965. However, the development of drugs that displayed a selective type of antidepressant activity underscored the very complexity of mental depressive illness and afforded a greater understanding of the fact that mental depression is not a single disease entity but requires individualized therapy and the availability of drugs capable of coping with the various facets of this mosaic disease syndrome.

Other major developments demonstrated the influence of the antidepressant drugs on catecholamine metabolism, uptake, storage, and intracellular binding. These findings shed new light on the mechanism of action of these drugs pointing to a possible etiology of a breakdown in central chemical homeostasis.

Well-controlled clinical investigations brought into sharper focus the need for both stimulant and tranquilizing antidepressant agents, particularly where depressant symptoms were an overt expression of an underlying psychotic illness which was often exacerbated by the "stimulant" type of antidepressant.[1,2] The introduction of monodemethylated imipramine and amitriptyline derivatives was a step in the direction of achieving greater selectivity of action.[3,4,5,6]

B. The MAO Inhibitors

1. New Structures



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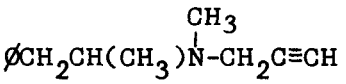

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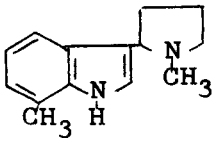

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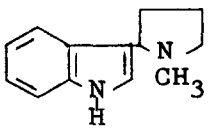
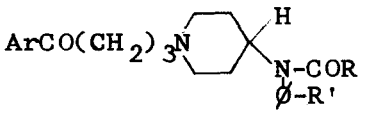


(IVa)



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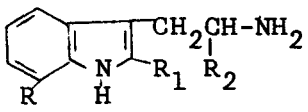


(VI)

R;R_1;R_2=lower alkyl groups

Ar = phenyl or substituted phenyl

R = alkyl, cycloalkyl, heterocyclic amine

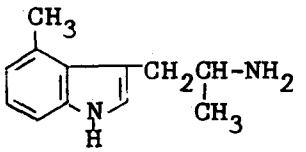
Structures I [7] and II are modifications of pargyline (Eutonyl™). Knoll et al.[8] describe structure II as being "an acute psychostimulant" and "a chronic psychic energizer". Its MAO inhibitory potency in vitro is said to be 200 times that of Niamid™. Unlike pargyline, this agent is a potent central stimulant comparable to amphetamine in potency.

Structure III, a phenoxy analog of tranylcypromine was one-third as potent as the latter drug and twice as long-lasting.[3,9] In rats, III displayed moderate anorexigenic and increased locomotor activities.

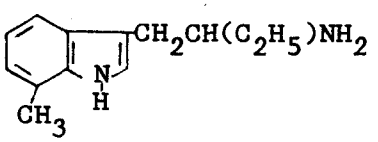
As an MAO inhibitor, compound IV was approximately one-hundredth as potent as pheniprazine (α-methylphenethylhydrazine). As a group, these compounds displayed a variety of pharmacologic effects: CNS stimulation, tryptamine and nicotine antagonism and suppression of fighting mouse behavior.[10] The data presented do not allow a conclusion concerning their potential as a "lead" in the area of antidepressants or CNS stimulants. The series is interesting, however, because of the dualistic type of psychotropic action (excitant and tranquilizing) and bears further watching.

Compound V is another structure displaying a multitude of pharmacologic actions.[11] Both neuroleptic and "potent" in vitro MAO inhibitory properties are ascribed to this series. The clinical utility of this structural group in mental depression remains in doubt.

Structure VI illustrates variations on the theme of Monase™ (etryptamine).[12] Compound VII has been investigated clinically by Azima[13] who found it to be an effective antidepressant. The 7-methyl-α-ethyltryptamine (VIII) is said to be superior as an MAO inhibitor to etryptamine, both in vitro (ten times) and in vivo (two to four times).[14] The clinical utility of this agent in antidepressant therapy has not been revealed.



(VII) MP-809



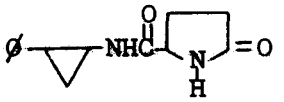
(VIII)

2. New Developments on Older MAO Inhibitors

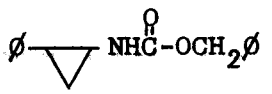
The hypertensive crises produced by tranylcypromine in the presence of tyramine-rich foods [15,15a,15b,15c] has restricted the application of this valuable agent to hospital use. While other MAO inhibitors evoke similar responses in tyramine-treated rats,[16] the intrinsic sympathomimetic activities of tranylcypromine presumably enhance its potency in this regard.[17]

Efforts to overcome the peripheral side effects of the MAO inhibitors have taken two directions: (1) Masking of the free amino group of tranylcypromine by a suitable acyl radical[18,19]

(structures IX and X) which would not impede the drug's passage

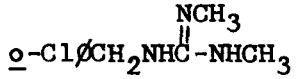


(IX)

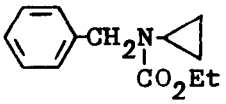


(X)

across the blood-brain barrier nor form so stable an amide linkage as to preclude enzymatic cleavage to the free amine at the target site. (2) Blockade of the peripheral cell receptors of MAO by a reversible MAO inhibitor with greater receptor affinity than the therapeutically active MAO inhibitor and an inability to penetrate the blood-brain barrier. In this way, the therapeutic MAO inhibitor will be forced into the central nervous system or metabolized to an easily excretable metabolite. Horita[20] has succeeded in demonstrating the utility of this concept. Premedication of the rat with a reversible MAO inhibitor, BW 392C60 (XI), followed by treatment with pheniprazine blocked brain MAO completely, but maintained high MAO activity in the liver and other peripheral organs. Pheniprazine and pargyline could be effectively antagonized, but not tranylcypromine or iproniazid. Compound MO 1255 (XII) has been claimed to be an active antidepressant devoid of cardiovascular side effects.[21]



(XI) BW 392C60



(XII) MO 1255 (encyprate)

Modaline™ (2-piperidino-3-methylpyrazine) has been shown by Rider[22,22a] to produce gastric antisecretory effects comparable to atropine. The drug has properties common to both MAO inhibitors and the imipramine-type agents. As an MAO inhibitor, it is four to six times as potent as phenelzine, but unlike the latter, produces rather severe orthostatic hypotension in man.[22a]

3. Mechanism of Antidepressant Action of MAO Inhibitors

At the present state of our knowledge, the mechanism of the antidepressant action of the MAO inhibitors is thought to be intimately tied to brain catecholamine levels. Schildkraut [23] has summarized the evidence favoring the involvement of catecholamines in transmitting the effects of the MAO inhibitors. Spector [24] showed that during selective depletion of norepinephrine and dopamine, the MAO inhibitor, pargyline, was unable to reverse reserpine or tetrabenazine depression until brain catecholamine levels had reached 50% of pre-drug levels.

Recently, Ingvarsson [25] has reported dramatic remissions of long-standing drug resistant depressions following the administration of 50 mg. of DOPA every other day. Marked improvement

was seen within a few hours. Patients also afflicted with asthma and Parkinsonism experienced complete relief of their symptoms from the DOPA treatment. Relapse occurred on discontinuance of DOPA therapy.

The inhibition of NE biosynthesis by blocking the rate-limiting step with α-methyltyrosine resulted in impairment of motor activity and sedation in animals and man[26,27] - Clinical improvement in the depressed patient correlated well with the degree of MAO inhibition.[28,29] Recently, Pletscher[30] has presented evidence that the MAO inhibitors may block the uptake of released NE into storage granules, thereby producing an increased concentration of NE around the adrenergic synapses.

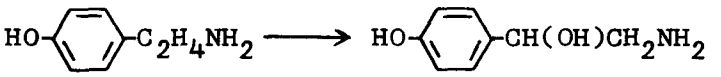
4. Conclusions

a. Potent, irreversible inhibition of brain MAO is a primary pre-requisite for effective antidepressant therapy.

b. The close temporal relationship between the onset of MAO inhibition and antidepressant effects lends further support to the hypothesis that "free" NE, protected from oxidative metabolism, may be implicated in mediating the antidepressant effects of the MAO inhibitors.

c. Clinically, the MAO inhibitors exert optimum antidepressant effects in reactive and neurotic depressions. Activated, psychotic or endogenous depressions were less susceptible to MAO inhibitory therapy.[25,31]

d. The "False Neurochemical Transmitter" theory promulgated by Kopin et al.[32] to explain the sympathetic blocking properties of the MAO inhibitors, must be seriously considered also with respect to the central properties of these agents. In essence, this hypothesis proposes that MAO inhibition will produce an accumulation of sympathomimetic metabolites which are normally not present in the body, but have sufficient affinity for the adrenergic receptor sites to displace the regularly present neurotransmitters (e.g., norepinephrine, dopamine) from sympathetic nerve endings. Furthermore, sympathetic nerve stimulation will release these agents in the same manner as it does NE; however, the resultant effect will be greatly diluted, since the false neurotransmitters have distinctly weaker adrenergic properties than NE or dopamine. Normally, tyramine is rapidly metabolized by MAO so that little, if any, octopamine is formed. In the presence of MAO inhibitors, however, significant amounts of octopamine are produced.



(XIII) Tyramine (XIV) Octopamine (False Neurotransmitter)

5. Review Articles

For further detailed information regarding adverse clinical side effects,[33,36] comparative clinical efficacy [34,35,36,37] and

possible mechanism of action [38,39,40] of the MAO inhibitors, the reader is referred to the cited review articles.

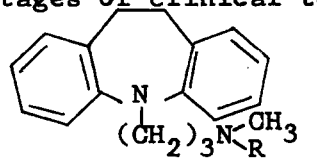
Structure-activity, biochemical, pharmacological and clinical pharmacological data on both hydrazine [39,40] and non-hydrazine MAO inhibitors [41] have been covered extensively in the respective references.

Schildkraut's paper [38] is of special interest, since it provides much of the supporting evidence for the "Catecholamine Theory" of the antidepressant drug action.

C. The Thymoleptic Agents (Tricyclic Antidepressant Drugs)

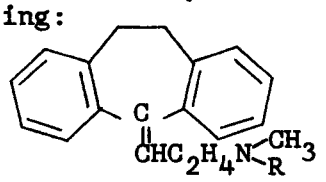
1. Introduction

Listed below are the structures of the tricyclic antidepressants which are either commercially available or in advanced stages of clinical testing:



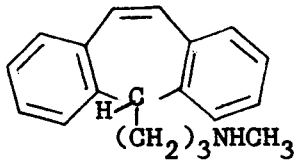
(XV)

R=CH_3: Imipramine
R=H: Desipramine



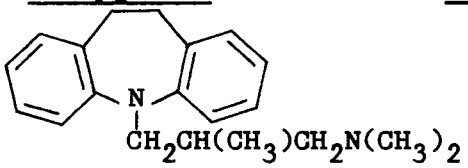
(XVI)

R=CH_3: Amitriptyline
R=H: Nortriptyline



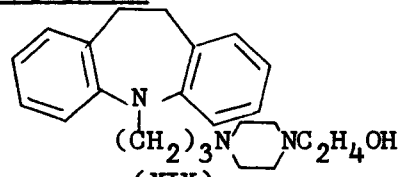
(XVII)

Protriptyline



(XVIII)

Trimipramine



(XIX)

Opipramol

Kuhn's discovery[42] of the antidepressant effects of imipramine constituted a major break-through in the treatment of mental depression. An excellent and comprehensive review by Häfliger and Burckhardt[43] covers the chemistry, pharmacology, and clinical effects of this drug group until 1963.

2. Evidence for Enhancement of Adrenergic Responses by the Thymoleptic Drugs

The original pharmacologic spectrum of imipramine gave no hint of its potential activity as a novel clinical antidepressant; rather, the drug behaved like a mild tranquilizer in animals. Sigg[44] was the first to discern pharmacologic differences between imipramine and chlorpromazine on the basis of enhancement of certain adrenergic responses to exogenously administered NE by the former drug. Sigg ascribed this action of the drug to a "sensitization of the adrenergic receptors."

Kaumann et al.[45] demonstrated an increase in cardiac rate and force of contraction with desipramine which would be blocked by DCI. Ursillo and Jacobsen[46] working with the isolated

vas deferens achieved marked quantitative differences between minor and major antidepressants. Thus, desipramine had 50 - 100 times the potency of methylphenidate. Amitriptyline, chlorprothixene, chlorpromazine produced only inhibition of NE in this test. Catecholamine-induced hyperthermia in rats was potentiated by imipramine.[47] Imipramine and desipramine, in small doses, enhanced amphetamine stimulant and hyperthermic effects. Opipramol produced only a slight potentiation and clinically, this drug is less of an antidepressant than an anxiolytic agent.

3. Mechanism of NE Potentiation by the Thymoleptic Agents

One of the major routes of inactivation of freshly released (from cellular binding sites) NE is re-uptake by the cell.[48a] Glowinski and Axelrod[49] devised a technique capable of determining brain levels of exogenously administered (by intraventricular injection) tritiated NE. The exogenous NE is taken up and retained by the sympathetic nerve endings in the brain and behaves biochemically like the endogenous neurotransmitter. These investigators were able to show that only clinically active antidepressant drugs (imipramine, desipramine and amitriptyline) were capable of reducing the uptake of tritiated NE in the rat brain. A close structural analog of imipramine, inactive as an antidepressant, was devoid of any activity on NE uptake. The authors suggest that the ability of clinically active antidepressants to block the re-uptake of freshly released "free" (active) NE by the cerebral tissues may account for the mechanism of their antidepressant action. Iversen[50] working with the isolated rat heart, found desipramine to be 100 times more potent as an inhibitor of NE uptake than chlorpromazine.

In the isolated perfused cat spleen, Thoenen et al.[51] showed that NE output resulting from sympathetic stimulation was increased and the inactivation of exogenously administered NE delayed in the presence of previously administered imipramine or protriptyline. Haefely et al.[52] concluded from their studies with the active thymoleptic drugs, that such antidepressants exerted at least three types of activity at the peripheral adrenergic synapses: (1) a sympathomimetic effect, presumably due to release of active NE from its binding sites, (2) inhibition of re-uptake of NE by the storage sites of sympathetic nerve endings, and (3) a sympathicolytic or noradrenolytic effect in higher doses. The desmethyl derivatives were uniformly more potent in intensifying the sympathetic effects of neuronally released NE.[51]

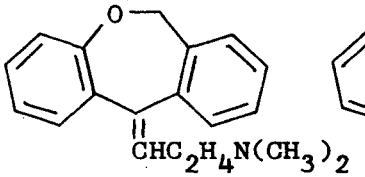
4. Interaction of Thymoleptics with Reserpine, Tetrabenazine, or Benzoquinolizines

The thymoleptic agents respond similarly to the above three catecholamine "releasing" drugs. Sulser and Soroko[53] have shown that the reversal of reserpine sedation depends on the availability of NE stores and the rate of release of NE from its binding

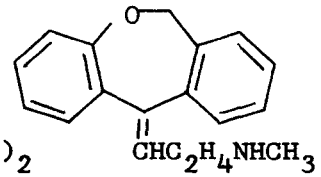
sites. In animals, selectively depleted of their NE by α-MMT, the thymoleptic drugs were unable to reverse the reserpine-induced depression. There was a definite temporal relationship between brain levels of desipramine and reversal of the sedative response of the benzoquinolizines.[54] Hence, the authors concluded that the reversal of reserpine stupor by the thymoleptic drugs is dependent on rapid and copious release of brain catecholamines.

5. Anticholinergic Effects of Thymoleptic Drugs

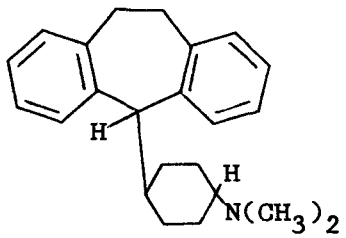
An alternate or additional mechanism of antidepressant drug action which would implicate blockade of central cholinergic responses had been proposed originally by Biel et al.[55] Thymoleptic drugs, such as imipramine and desipramine have been shown by Sulser et al.[56] to antagonize all the central parasympathomimetic effects of reserpine (increased salivation, muscular rigidity, hunchback posture and blepharospasms), even in catecholamine-depleted animals. The central anticholinergic activity of oxygen isosteres of amitriptyline and nortriptyline (XX and XXI) was demonstrated[57] by their antagonism to the cholinesterase inhibitor, diethyl p-nitrophenyl phosphate (Paraoxon) and emetic effect in pigeons. Increased peripheral anticholinergic effects were displayed by the centrally more potent diastereoisomer (B) of structure XXII. Diastereoisomer (A) was considerably less active as an anticholinergic and central stimulant.[58]



(XX)



(XXI)



(XXII)

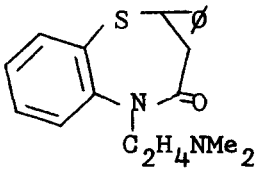
Monro et al.[59] investigated a series of tricyclic antidepressant drugs and found a positive correlation between potent CNS properties and anticholinergic potency in animals.

Giarman and Pepeu[60] demonstrated that only centrally active (animals and man) basic glycolate esters caused a reduction in brain acetylcholine (AcCh) levels of the rat. The authors suggested as a likely mechanism, interference of the psychotropic glycolates with storage of brain acetylcholine resulting in reduced uptake of newly synthesized AcCh.

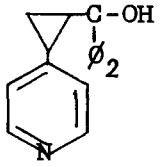
Conclusion. On the basis of presently available experimental evidence, one would have to conclude that the mechanism of action of the tricyclic antidepressant drugs may be due (1) to an interference with cellular binding of brain catecholamines, presumably NE, thereby producing increased concentrations of catecholamines at the central adrenergic synapses, and (2) a lowering of brain AcCh levels which would tend to enhance the overall antidepressant effect.

D. Newer Antidepressant Drugs

Two drugs which represent structural departures from the currently marketed antidepressants are shown below (XXIII,XXIV):



(XXIII) SQ 10,496 (Thiazesim)



(XXIV) IN 1060

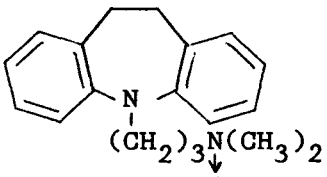
Compound XXIII displayed activity only in "septal" rats by suppressing the rage reaction and hyperirritability.[61] Unlike other antidepressants, XXIII did not produce a reserpine reversal. In man, preliminary data indicate it to be a rapidly acting antidepressant drug.[62]

As to compound IN 1060, Sletten et al.[62a] concluded that the pharmacologic profile of this compound resembles that of imipramine with respect to (1) activation of psychotic processes, (2) increase in spontaneous motor activity in mice, (3) reversal of reserpine-induced ptosis and depression in rats, (4) antagonism to Ditran-induced behavior in dogs, and (5) potentiation of epinephrine and NE pressor response of systolic blood pressure in man.

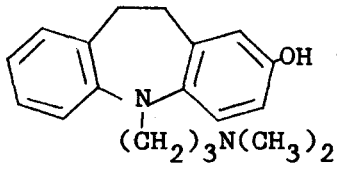
E. The Significance of Imipramine Metabolites

Kuhn[63] has tried to correlate the urinary excretion of imipramine metabolites with clinical improvement.

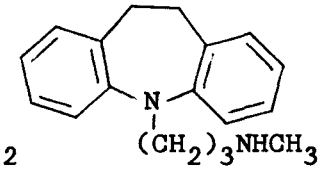
Imipramine Metabolites



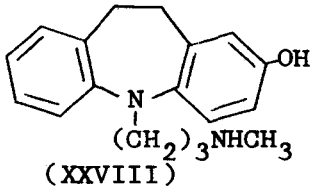
(XXV)



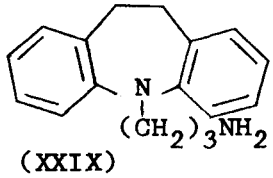
(XXVI)



(XXVII) desipramine



(XXVIII)



(XXIX)

While a strict correlation proved difficult, clinical improvement normally became evident following the maximum urinary excretion of metabolite XXVIII which usually occurred between the sixth and fifteenth day of therapy and was preceded by accumulation of

metabolite XXVII, desipramine. Kuhn suggests that the formation of metabolite XXVIII is dependent on the presence of significant quantities of XXVII whose formation may be the rate-limiting step in generating metabolite XXVIII. The parallelism in the temporal relationship between the onset of the antidepressant effect following imipramine and the appearance of maximal amounts of XXVIII in the urine would explain the more rapid onset of action (two to four days) of desipramine.[64] Sulser et al.[56] have shown that animal species which are unable to convert imipramine to desipramine are also incapable of counteracting reserpine or benzoquinolizine-induced sedation with imipramine.

F. Clinical Properties of Antidepressant Drugs

A number of review papers have appeared concerning the clinical pharmacologic[23] and clinical properties of the MAO inhibitory and thymoleptic agents.[65,66,67,68,69,70] Ayd [71] has published a compilation of his clinical experiences with amitriptyline during a six-year period. Space permits only a summary of the general conclusions which have been reached by these authors:

1. Mental depression is a multifaceted disease syndrome with varying types of etiology. It may be primary or secondary (i.e., resulting from other mental disturbances such as severe anxiety or an underlying psychosis). It may be reactive (i.e., in response to an environmental stress) or endogenous (i.e., arising from within the patient due to a faulty personality make-up). It is often subject to a spontaneous remission.

2. With respect to the comparative efficacies of the antidepressants, the recent review paper by Wechsler et al.[69] is probably most pertinent. The authors' conclusion may be summarized as follows:

a. Variability - Reported effectiveness for imipramine, amitriptyline, EST, iproniazid, phenelzine, isocarboxazid and nialamide often ranged from 0 to 100%. Amitriptyline had the least variability (32 to 79%).

b. Improvement Rate - Average improvement rate for patients on imipramine, amitriptyline, and isocarboxazid was 65%, whereas the range for iproniazid, phenelzine, and nialamide was 40 to 49%. ECT ranked the highest with 72% improvement in depressive symptoms and placebo therapy the lowest (23%).

c. Relative Chronicity of Depression - Depressions of recent origin afforded a much higher improvement rate than chronic depressions. For phenelzine, the rate decreased from 56 to 8%, and for imipramine and amitriptyline from 69 to 45% and 66 to 32%, respectively.

3. Anergic (lethargic) depressions responded best to the "stimulant" type of antidepressant (imipramine, desipramine, protriptyline), whereas agitated or anxiety depressions improved most with the "sedative" type of antidepressant drug (amitriptyline, nortriptyline). All the antidepressants exacerbated psychotic symptoms; however, amitriptyline, having a therapeutic spectrum in between chlorpromazine and imipramine, was the smallest offender in this regard. The recent combination (Triavil™, Etrafon™) of a potent antidepressant (amitriptyline)

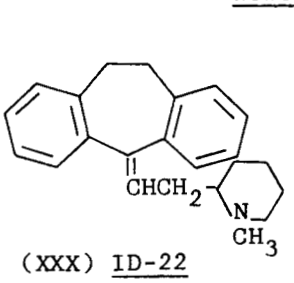
with a potent tranquilizer (perphenazine, Trilafon™) represents an attempt to deal more effectively with depressions accompanied by anxiety, agitation or schizophrenic symptomatology. The preliminary reports appear favorable.[72,73,74]

4. The desmethyl derivatives of imipramine (desipramine, Norpramin™, Pertofrane™) and amitriptyline (nortriptyline, Aventyl™) provided a faster onset of action (two to four days) and a lessened intensity of parasympatholytic and adrenolytic side effects.[75,76,77] Nortriptyline proved to be a valuable adjunct in the treatment of gastro-intestinal disturbances.[78]

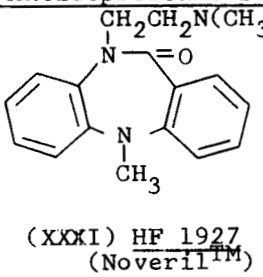
G. Clinical Properties of Some Newer Antidepressant Agents

More radical structural departures from the imipramine and amitriptyline-type of antidepressant agent are shown below:

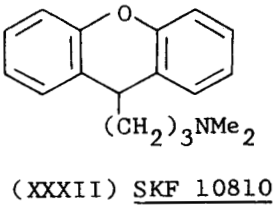
Newer Antidepressant Structures



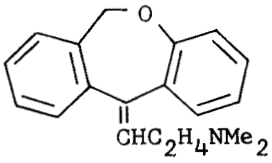
(XXX) ID-22



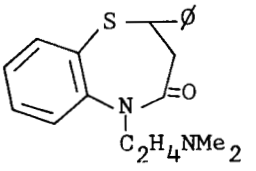
(XXXI) HF 1927 (Noveril™)



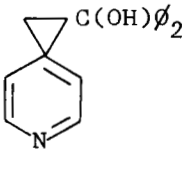
(XXXII) SKF 10810



(XXXIII) P-4599



(XXXIV) SQ 10,496 (Thiazesim)



(XXXV) IN 1060

Preliminary clinical data indicate that ID-22 is a satisfactory antidepressant endowed with both stimulant and tranquilizing properties (depending on the patient); side effects included orthostatic hypotension, dry mouth, confusional symptoms, and nocturnal anxiety.[79] Compound HF-1927 was effective in the treatment of inhibited and agitated endogenous depressions; the incidence of psychotomimetic and cardiovascular side effects was high.[80,81,82]

Freeman et al.[83] have described the rapid (three days) onset of thiazesim. After two weeks' treatment with this agent, 70% of the patients could be discharged. Unfortunately, the discharge rate was quite comparable in the placebo-treated group. The imipramine-like properties of IN 1060 in animals and man have been discussed in Section I-D. In preliminary clinical studies, the drug exerted beneficial effects in anergic schizophrenics and a small number of depressed patients.[84]

H. The Anticholinergic Antidepressants

The clinical psychotomimetic and antidepressant properties of Ditran have been reviewed by Biel.[85] A more recent study by Davis et al.[86] found this drug a "worthwhile, safe, and effective" antidepressant when tested in 78 patients over a period of two and one half years. Improvement became apparent after five to six treatments spaced two to three days apart. Tachyphylaxis to the psychotomimetic effects were quite pronounced in all patients. Prolonged remissions of illness appeared possible. Reactive, psychotic, and schizo-affective depressions responded best to Ditran, whereas schizophrenic and psychoneurotic depressions proved quite resistant to this type of therapy.

In a well-controlled study by Fink et al.[87] the addition of an anticholinergic-antiparkinsonism agent, procyclidine, to chlorpromazine produced an antidepressant effect comparable to imipramine.

Centrally active anticholinergic agents, either alone or in combination with a tranquilizer, deserve further evaluation as potential antidepressant drugs.

Final Conclusions

The principal developments in the field of antidepressant drugs during 1965 may be summed up as follows:

1. An increased understanding of the possible mechanisms of antidepressant drug action was gained through the study of their effects on central adrenergic and cholinergic neurotransmitters.

2. The availability of several drugs with differential types of useful antidepressant activity served to demonstrate that "mental depression" is a "catch-all" phrase which encompasses several disease syndromes of varying etiologies and requiring antidepressant drugs with either stimulant, tranquilizing, or antipsychotic properties.

3. Some progress was made toward achieving a faster onset (two to four days) of action and a lessening of side effects with the desmethyl derivatives of active thymoleptics.

4. Advances in methodology of animal testing for antidepressant activity revealed definite qualitative differences between the antidepressant and tranquilizing agents.

5. On the basis of the mechanism of action of various effective antidepressant drugs, one could conceive of a biochemical etiology for mental depressive illness founded on the inability of the central nervous system to (a) elaborate sufficient amounts of neurotransmitter substance (possibly norepinephrine) (b) release adequate quantities of "active" neurotransmitters from cellular binding sites to the adrenergic synapses or (c) prevent a too rapid metabolic destruction of the critical neurotransmitter substance(s).

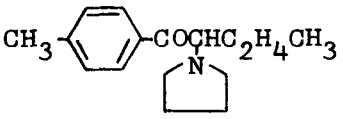
6. Clinical evaluation of antidepressant drugs still poses a major problem due to the complex nature of the disease, the high incidence of favorable placebo responses and spontaneous remissions.

II. Central Stimulants

The central mechanism of action of amphetamine still remains in dispute. Since central catecholamine depletors (reserpine, α-MMT) failed to block amphetamine-induced motor stimulation, several authors have felt that amphetamine acts directly on the CNS.[88,88a,88b,88c,88d] On the other hand, Stein[89] points out that amphetamine's facilitating effect on intracranial self-stimulation in rats was blocked by reserpine and potentiated by MAO inhibitors. Hence, he concluded that the central stimulant effects of amphetamine were mediated by catecholamine release.

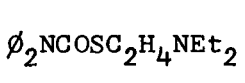
A novel mechanism has now been proposed by Weissman et al.[89a] which is based on the experimental finding that tyrosine hydroxylase inhibitors (e.g., α-methyltyrosine) which block the biosynthesis of dopamine and NE, also inhibit all the CNS effects exerted by amphetamine and amphetamine-like drugs (methamphetamine, phenmetrazine). Hence, the authors conclude that the central responses induced by amphetamine require a "critical level of NE at the receptor" and "that this level derives from a functional pool of norepinephrine in the CNS highly susceptible to blockade of norepinephrine biosynthesis at the tyrosine hydroxylase step", which is the rate-limiting step in catecholamine biosynthesis.[89b] On the other hand, α-MMT which is a potent catecholamine-depleting agent, but does not interfere with NE biosynthesis, fails to antagonize the central effects of amphetamine. In essence, amphetamine may require the presence of freshly synthesized, readily available NE to produce its characteristic CNS effects.

Clinical studies on a novel sympathomimetic ketone (F-1983) revealed that this substance was capable of increasing drive and spontaneous activity in a group of chronically depressed and psychotic patients.[90]

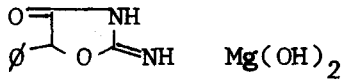


(XXXVI) F-1983

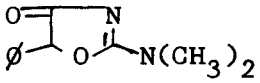
Central stimulant and antitremorine effects were displayed in animals by a diphenylthiocarbamate (XXXVII).[91] A mild central stimulant (XXXVIII) was shown by Glasky and Simon[92] to promote the biosynthesis of RNA in rat brain and by Plotnikoff[93] to facilitate learning by increasing memory and retention of learned behavior. This property was not shared by other central stimulants.



(XXXVII)



(XXXVIII)



(XXXIX)

A structurally similar compound (XXXIX) has been investigated by Greenblatt and Osterberg.[94] The pharmacologic spectrum of XXXIX lies between amphetamine and imipramine. The compound is

a central excitant with anorexigenic properties. The latter are more pronounced and longer lasting than those of amphetamine. Tolerance did not develop to either effect. The drug displayed only minimal cardiovascular effects and was devoid of analeptic properties. Like imipramine, it delayed the onset of reserpine depression and inhibited tetrabenazine depression. It protected mice from maximum seizures and prolonged barbiturate hypnosis.

III. The Hallucinogens

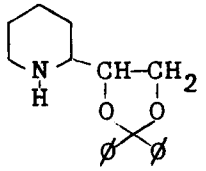
Most drugs capable of penetrating the CNS have structural relatives which will produce psychotomimetic effects. This applies to the analgetic, antidepressant, anticholinergic, anesthetic, sympathomimetic, serotonergic and tranquilizing drugs. It illustrates perhaps more than anything else the fine line which we are straddling between normal and pathologic emotional behavior and underscores the sensitive chemical balance necessary for the maintenance of central homeostasis and presumably "normal" behavior. For background material, the reader is referred to the following review articles:[95,96,97]

A most comprehensive review on LSD_{25} has been published by Hoffer.[98] The social and therapeutic implications concerning the use of LSD_{25} have been discussed by Cole and Katz,[99] Savage and Stolaroff,[100] and McGlothlin and S. Cohen.[101]

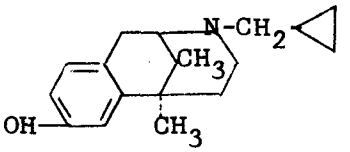
Structure-activity relationships with regard to the central stimulant and psychotomimetic properties of the basic glycolate esters have been presented by Biel et al.[102] and Abood and Biel.[103]

A. Analgetics

Psychotomimetic properties have been reported for dexoxadrol[104] and cyclazocine[105] in man. In animals, both drugs were devoid of analgetic activity but displayed morphine antagonism.



(XL) Dexoxadrol



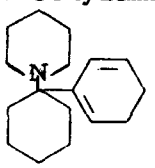
(XLI) Cyclazocine

B. Anesthetics

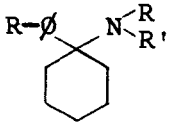
As an anesthetic, phencyclidine (Sernyl™) is quite unique from the standpoint of high potency, lack of respiratory and circulatory depression or disturbance of cardiac rhythm both in animals[106] and man[107]. Its psychotomimetic properties have been reviewed by Davies and Beech.[108]

The similarity in sympathomimetic properties of phencyclidine, desoxyephedrine and cocaine prompted Chen et al.[109] to speculate on a central adrenergic mechanism of action, even though the insertion of a methylene group between the phenyl and cyclohexyl

ring to simulate a β-phenethylamine abolished all activity. A simple, specific and quantitative test for the assessment of cataleptic activity of phencyclidine-type compounds in pigeons has been developed by Chen.[110] The cataleptic effect is ascertained by the loss of righting reflex without "head drop" over a wide range of dosages. A structure-activity study was conducted by Maddox et al.[111] The most potent cataleptic agents were those where R = H, methyl or methoxy and N(R,R') was piperidino, pyrrolidino, 3-methylpiperidino, β-methoxyethylamino, methylamino or ethylamino.



(XLII) Phencyclidine (Sernyl™)



(XLIII) Phencyclidine Derivatives

C. Adrenergic Agents

The proponents of an abnormal amine metabolism in schizophrenic patients received further support for their speculation from the experimental finding of Friedhoff and Van Winkle [112] that 3,4-dimethoxyphenethylamine (DMPEA) was excreted in the urine of schizophrenic patients, but could never be isolated from the urine of normal patients. Bourdillon et al.[113] were able to demonstrate that when the schizophrenic population was further subdivided, certain groups showed high concentrations of this amine in the urine. For an up-to-date list of references on this controversial subject, the reader is referred to an Editorial.[114]

Friedhoff and Van Winkle[115] further found that liver homogenates obtained from biopsies of schizophrenic patients were capable of O-methylating both 'OH' groups of dopamine, whereas those obtained from normal subjects were unable to convert dopamine to DMPEA. In cats, DMPEA produced a catatonic effect similar to that of mescaline.[116,117]

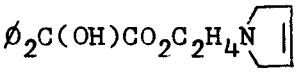
D. The Central Anticholinergics

It had previously been concluded by Biel[118] that potent anticholinergic activity was a primary pre-requisite for inducing psychotomimetic effects both in animals and man, but that not every potent anticholinergic drug need necessarily be a psychotogenic agent. Giarman and Pepeu[119] reported that rat brain levels of acetylcholine were reduced by the psychotomimetic glycolate esters, but not by non-psychotogenic anticholinergics of similar structure. A single injection of 0.5 mg/kg of scopolamine to rats which had been trained for a short period, produced an amnesic effect of learned performance which correlated well with the decrease in total rat brain AcCh levels.[120] Both effects were abolished by eserine and amphetamine. On the other hand,

overtrained rats experienced no amnesic effects, even though brain AcCh levels were decreased to the same extent. The authors concluded that only recent memory may be cholinergically mediated. In adequate doses, scopolamine disrupted severely both the acquisition and retention of passive avoidance response in rats.[121]

In a recent paper by Deutsch et al.[122] the injection of the anticholinesterase drug, diisopropyl fluorophosphate, into the hippocampi of rats, 30 minutes after escape learning, produced a partial amnesia lasting for five days.

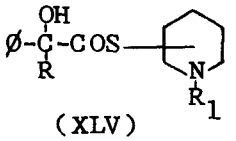
Centrally, scopolamine had ten times the activity of atropine, whereas in the periphery, the ratio was closer to two to one, respectively.[123] The compound (Ro 3-1172) was reported by Parkes [123] to have 50 times the CNS activity of atropine.



(XLIV) Ro 3-1172

Short-lasting psychotomimetic effects (two hours) were induced in humans by N-allylnoratropine (5 mg/man). In the periphery, this compound only had 1/16 the autonomic activity of atropine.[124] The interaction between phenothiazine tranquilizers in small doses and the central anticholinergics, atropine, scopolamine, and Ditran (JB-329) was studied by Gershon et al.[125] Chlorpromazine, at a dose of 0.1 mg/kg, potentiated the effects of 0.05 mg/kg of Ditran producing a comatose-like state in human subjects. Imipramine did not potentiate the action of Ditran. Hence, this test could be applied in dogs to differentiate an antidepressant from a tranquilizing drug.

Buehler et al.[126] synthesized a number of thiol analogs of basic glycolate esters which were considerably weaker as psychotomimetic agents than the corresponding oxygen analogs.



R = Ø, cycloalkyl

R_1= CH_3, C_2H_5

(XLV)

The influence of stereochemical factors on anticholinergic psychotomimetic activity of some piperidyl glycolates have been discussed by Gabel and Abood.[127]

Summary

Present evidence suggests that mental depression may be the cause or consequence of a breakdown in central chemical homeostasis, i.e., an imbalance between two mutually antagonistic central neurotransmitter systems, which may possibly be adrenergic-like and cholinergic-like in character.

Chemical correction of this imbalance through drug treatment has afforded a means of accomplishing remissions in acute, non-psychotic depressions.

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

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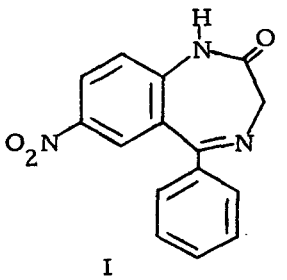
Introduction - The classification of a given drug according to the pharmacological actions listed in the title (plus tranquilizers discussed in Chapter 1) is recognized as arbitrary and dependent upon dosage being used. The following discussion is divided more on the basis of established or projected clinical utility than on the basis of widely different pharmacological properties or chemical structure.

Some years ago, Chen and Portman[1] undertook a quantitative approach to the problem. They determined the CD_{50}'s of a convulsant in mice pretreated with graded doses of a depressant. Plotting these values gave a graph showing two points of inflection, and three corresponding straight lines were constructed. The slopes of these lines were interpreted as representing the sedative, hypnotic and anesthetic potencies of the depressant. The intersects represented minimal hypnotic and anesthetic doses. The authors concluded that this approach was useful in indicating that a given depressant drug might be more useful as a hypnotic, an anesthetic or as a sedative.

Sedatives and Hypnotics - Several articles describe the favorable results obtained with nitrazepam, Mogadon®, RO 4-5360 (I). The pharmacology was thoroughly investigated by Randall and coworkers[2]. They postulate that the sleep-inducing effect is due not to a direct effect on the arousal system, but to a reduction of stimuli acting on it. Metabolic studies showed the unchanged drug as well as its 7-amino and 7-acetylamino derivative in human plasma and urine.

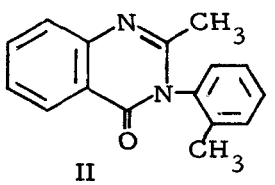


I

Clinical studies by Wyss and Mäder[3] in 200 patients gave good results in onset and duration of sleep at doses of 5 to 10 mg. At higher doses (up to 200 mg.), tolerance was excellent. They state that the mechanism of action differs from known hypnotics. Lanoir, Dolce and Chirinos[4] found that in a battery of neurophysiological tests in cats, nitrazepam and diazepam showed the same type of activity in many respects, such as action on spontaneous rhythm, evoked responses, etc. Both are potent anti-convulsants. The major difference is that nitrazepam induces a lasting and profound sleep, while diazepam acts essentially as a relaxant and not as a hypnotic. Borck[5]

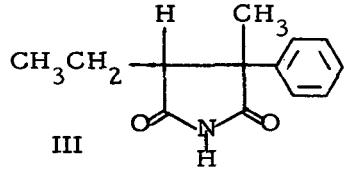
reported that the drug in doses of 5 to 15 mg. was a highly suitable hypnotic in psychiatric patients. Egert and Jahn[6] found it gave very good to good results in 75% of a wide variety of clinical cases. Particularly noteworthy were its new type of mechanism of action when compared with barbiturates, its low toxicity and its uselessness for suicidal purposes. In 40 children ranging in age from a few days to 11 years, Matthes[7] induced daytime sleep in 50% of the cases. Best results were obtained in infants up to 12 months of age.

About 10 years ago, the hypnotic, anticonvulsant and sedative properties of a series of 2-alkyl-3-aryl-4(3H)quinazolones were reported by Gujral and co-workers [8, 9] and confirmed by Boissier.[10] Recently, a series of 79 compounds of this type was examined by Leszkovszky, Erdely and Tardos[11] who reported that compound II showed the highest oral activity. Swift, Dickens and Becker[12] published a thorough pharmacological study of this compound in 1960. Clinical studies were published by several investigators [13, 14, 15, 16, 17] who compared methaqualone to glutethimide, chloral hydrate and cyclobarbital. A recent letter to the editor [18] cites four patients who consumed large amounts of the drug daily for extended periods, raising the question of physical dependence. In 1965, compound II was marketed as a hypnotic and sedative in the form of 150 mg. tablets as methaqualone (Quaalude®).



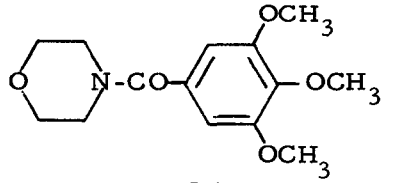
II

Fenimide is the non-proprietary name recently adopted[19] for 3-ethyl-2-methyl-2-phenylsuccinimide (III) which was described by Chen and Bass[20] as a sedative agent comparable to meprobamate and phenobarbital. It protects against stress-induced ulcers while meprobamate and phenobarbital do not.



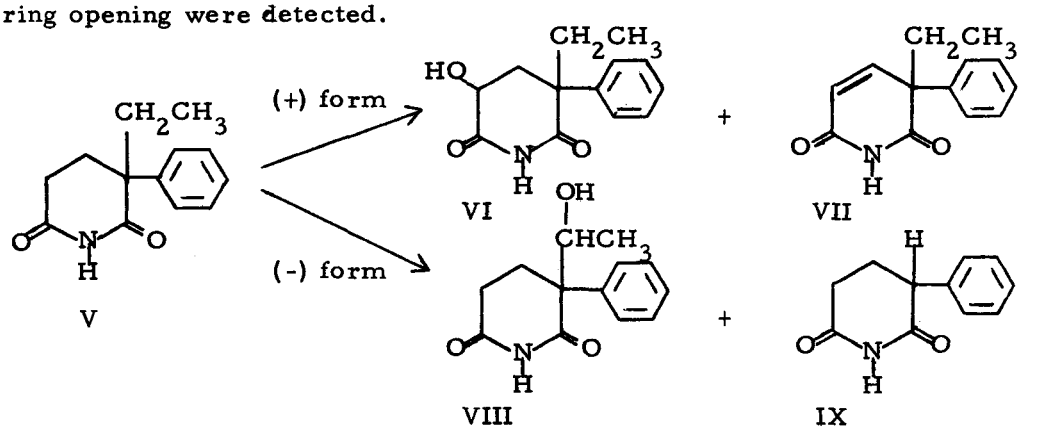
III

At the same time, the non-proprietary name trimetozine was adopted for 4-(3, 4, 5-trimethoxybenzoyl)morpholine. This compound (IV) was reported earlier by Vargha and co-workers [21, 22] as being selected from a large series of alkoxybenzamides for clinical trial as a neurosedative in Hungary. Of particular interest in their papers is the report of the marked influence on pharmacological activity of changes in position and/or nature of the alkoxy groups. Boissier and co-workers[69] stated that tests in animals show the compound to be a sedative but not a hypnotic even at toxic doses.



IV

Metabolic studies of the optical antipodes of glutethimide (V) (Doriden®) by Keberle, Riess and Hoffmann[23] are of especial interest since the authors showed stereospecific metabolism in dogs. The dextrorotatory form of V was hydroxylated in the ring to give mainly VI (isolated as the glucuronide) and VII, resulting from dehydration. The levorotatory form of V was hydroxylated in the ethyl group to give mainly VIII (isolated as the glucuronide) and IX, resulting from loss of acetaldehyde. Total recovery of these products was 96% of theory. No products resulting from ring opening were detected.



The sedative and hypnotic properties of thalidomide have been almost forgotten in view of its reported embryotoxic effects. Recent metabolic studies of thalidomide in rabbits, rats, mice and guinea pigs by Schumacher, Smith and Williams [24] showed that up to 12 hydrolysis products appear in the urine. These products are the same as those obtained from thalidomide in aqueous solutions at pH values above 6,[25] and can be accounted for by initial hydrolysis of the phthalimide ring followed by hydrolysis of the glutarimide ring. Earlier, the same group of investigators[26] had studied the relationship of embryotoxic activity and chemical structure in a series of compounds related to thalidomide and had concluded that a phthalimide group was important in determining teratogenic activity; and, in a recent article,[27] suggest that the reactivity of the drug towards certain natural diamines such as spermidine, putrescine, etc., may be of significance in relation to its biological properties.

Anti-convulsants - As in previous years, many compounds were tested and reported to be active as anti-convulsants during 1965. No new drugs for this use were marketed in the United States, and none marketed in other countries appear to offer considerable advantages over those in general use. A recent review by Millichap[28] discusses clinical and EEG indications, efficacy and toxicity of some 20 drugs in current use in this field.

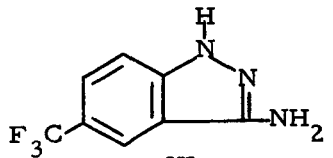
Central Muscle Relaxants - No new drugs in this field were introduced in the domestic market during 1965. Several reports were published of clinical trials of various compounds in severe spastic conditions. Bhargava and Srivastava[29] studied the anti-tetanus activity in cats of central muscle relaxants and found SQ10220, 3-(p-chlorophenyl)-3-hydroxy-2,2-dichloropropyl carbamate (X) to be the most potent and longest-acting of 11 compounds tested. Hendrickse and Sherman[30] found diazepam (Valium®) an effective muscle relaxant in children suffering from tetanus, although it was not very effective in controlling convulsive spasms.

X

Marsh[31] obtained excellent results in 10 of 26 severely cerebral-palsied children using oral doses of diazepam in a controlled study, confirming earlier reports of effectiveness in adults similarly affected. Encouraged by the results of Berman, Noe and Goodfield[32] using chlorzoxazone in cerebral-palsied patients abandoned to supportive care, Darienzo[33] used a combination of chlorzoxazone and acetaminophen and obtained a change from severe to mild symptoms in 12 of 15 children.

A metabolic study of chlormezanone (Trancopal®) in rat, dog and man by McChesney and co-workers[34] showed that the compound was excreted as such.

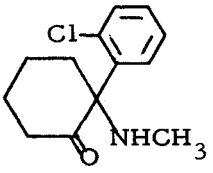
A review of benzazoles; chemistry, pharmacology and clinical application appeared.[35] SKF 13,436, 3-amino-5-trifluoromethyl-1H-indazole (XI) was reported by Santella and co-workers[36] to be a muscle relaxant in rats, cats, dogs, rabbits and monkeys.



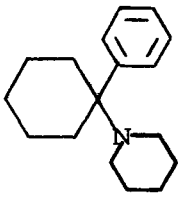
XI

Anesthetics (a) Inhalants - Data from thousands of patients were examined for possible relation between post-operative liver damage and anesthesia. Herber and Speckt[37] reviewed findings in about 20,000 cases and estimated that one case of hepatic necrosis may be expected in about 800 halothane anesthetics. They suggest that careful selection of patients should minimize liver necrosis in the continued use of this valuable anesthetic. Gingrich and Virtue[38] reviewed records of nearly 3800 cases using fluorinated hydrocarbons and over 20,000 cases using other inhalation agents as well as 48,000 non-surgical patients. They doubt that either halogenated hydrocarbons or other anesthetic agents per se have been of significance in the production of hepatic necrosis.

(b) Injection Anesthetics - McCarthy, Chen, Kaump and Ensor[39] studied the general anesthetic and other pharmacological properties of CI 581 (XII), an analog of phencyclidine (Sernyl®) (XIII) in eight species of animals and concluded that XII showed a lesser degree of CNS stimulation and of briefer duration than XIII. However, Domino, Chodoff and Corssen[40] found that in humans, XIII, like XII, was hallucinatory and that skeletal muscle tone was increased. They suggest the term "dissociative anesthetic" for this class of agents.



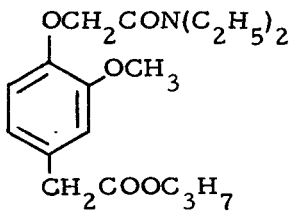
XII



XIII

The earlier reports by H. Laborit and co-workers[41] and by G. Laborit and co-workers[42] on the clinical use of sodium γ-hydroxybutyrate for sedation, hypnosis and general anesthesia stimulated several other authors to investigate the pharmacology of this compound.[43,44,45] Bessman and Skolnick[46] found that the depressant action paralleled the concentration in brain of the lactone and not of the anion. Recently, Winters and Spooner[47] suggest that a re-appraisal of the clinical usefulness of this drug may be in order, based on the epileptiform EEG patterns and grand mal seizures in cats after intraperitoneal administration of 0.7 to 1.0 g/kg.

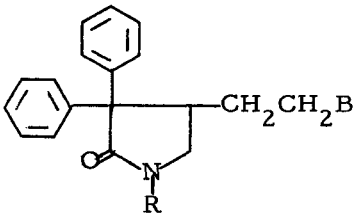
Propanidid (XIV) and a few other substituted phenoxyacetamides have been investigated further as very short-acting intravenous anesthetics. Gunner and co-workers[48] found propanidid useful in electroconvulsive therapy in 70 patients. They found the duration of unconsciousness averaged four minutes at a dose of 5 mg/Kg. and six minutes at a dose of 7 mg/Kg. They ascribe the brevity of action to hydrolysis of the ester group by esterases. Eisterer and co-workers[49] found the drug very suitable in 96 ambulatory patients requiring anesthesia of up to five minutes' duration. Hewitt, Hamilton, O'Donnell and Dundee[50] reported that, while propanidid showed a slightly higher incidence of venous thrombosis than thiopentone or methohexitone, the incidence was not enough to influence clinical acceptability.



XIV

Adjuncts to Anesthesia (a) Respiratory Stimulants - Lunsford and co-workers[51] synthesized a series of substituted pyrrolidinones (XV) and

studied their pharmacological properties. Some were stimulants of respiration and the CNS, and most of these showed pressor activity; others were depressants. One of the stimulants (XVI) was selected for further study.



XV

R = alkyl, cycloalkyl, benzyl
B = basic residue

XVI

R = ethyl
B = morpholino Dopram® Doxepram

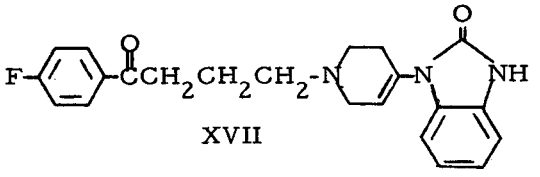
Evers, Malik and Dobkin[52] found doxepram to be as effective as d-amphetamine in shortening the recovery time in dogs given a standardized dose of thiopental; no untoward effects were observed. They also used the drugs as an intravenous infusion in 50 healthy female patients and found it effective for stimulating respiration (both tidal volume and respiration rate) without producing any appreciable circulatory changes.

Mauro and co-workers[53] studied 52 patients to whom doxepram was given and 23 controls; all were anesthetized with thiopental. A few seconds after a single injection of 0.3 mg/Kg. of doxepram, there was a marked increase in tidal volume to levels approaching control values.

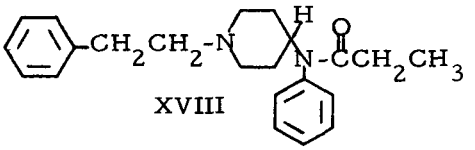
Noe, Borrillo and Greifenstein[54] injected 0.5 mg/Kg. doses into 20 patients anesthetized with (1) pentobarbital-pentothal, (2) nitrous oxide-oxygen or (3) halothane-nitrous oxide and found respiratory stimulation with more or less arousal in all three groups. Brief EEG changes were observed in five subjects and most cases showed moderate hypertension; otherwise, there were no side effects.

(b) Neuroleptanalgesics - Combinations of neuroleptic agents and narcotic analgesics have been widely used in surgical procedures with or without inhalation or injection anesthetics. Such combinations are referred to as neuroleptanalgesics and are usually considered as adjuncts to anesthesia. Proceedings of a Symposium held at Edinburgh were published.[68]

During 1965, some 20 to 30 articles appeared concerning clinical studies with Innovar®, a combination of the neuroleptic droperidol (XVII) and the analgesic fentanyl (XVIII). Contributions by the most active workers



XVII



XVIII

in the field are: Israel, Jansen and Dobkin;[55] Corssen, Chodoff, Domino and Kahn;[56] DeCastro, Mundeleer and Bauduin;[57] Gorodetzky and Martin;[58] Aubry, Denis, Keeri-Szanto and Parent;[59] and Gemperle and Buhler.[60]

Pharmacological studies using the combination of the two drugs were reported by Dobkin and co-workers;[61, 62] Chodoff and Domino;[63] and Canellas, Roquebert and Courtois.[64]

A combination of the two drugs was marketed during 1965 as Innovar®-Vet. Yelnosky and Field[65] investigated its use in six species of animals and found it most useful in dogs. Franklin and Reid[66] obtained good to excellent results in 564 out of 601 surgical procedures using various breeds of dogs. Mortelmans, Marsboom and Vercruyssee[67] reported highly successful results in 174 trials using primates and lower monkeys.

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Chapter 4. Analgetics--Strong and Weak

Louis S. Harris, University of North Carolina, Chapel Hill, N.C.

I. Introduction

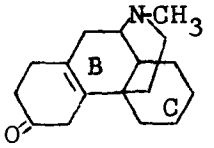
The past few years have witnessed a ferment in the field of analgetics. Serious questions have arisen to challenge our concept of pain as a specialized sensory system. Indeed we now take a more Aristotelian view of pain and speak of the "pain experience" which encompasses not only the sensation of pain but associated sensations such as touch and heat and feeling states such as fear and pleasure.

It is not surprising then that more and more drugs are becoming available for the treatment of pain which appear to have mechanisms which differ, sometimes quite markedly, from those of the classical agents. We now have strong analgesics which are devoid of morphine-like addiction potential and mild analgesics which are increasing in their pain relieving potency. It would appear that we are approaching closer and closer to the ideal analgesic agent. A recent monograph[1] presents an excellent summary of much of this work.

II. Strong Analgesics

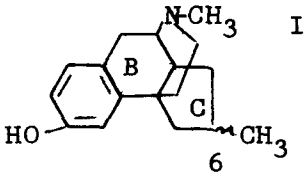
A. Morphine and Morphinans

There was little activity in this area in 1965. Pirkle and Gates[2] prepared the hydroaromatic analogs (I and II) of the potent analgesics l-3-hydroxy-N-methylmorphinan and the corresponding isomorphinan. The morphinan (I) with a cis-ring fusion was essentially inactive while the isomorphinan (II) was active



I B/C cis

II B/C trans



III B/C cis

IV B/C trans-6-oxo

only at near toxic doses. The secondary alcohol of I showed activity. Thus, reduction of the aromatic ring depresses analgesic activity and lends evidence to the importance of the planar ring for binding to or fit on the receptor.

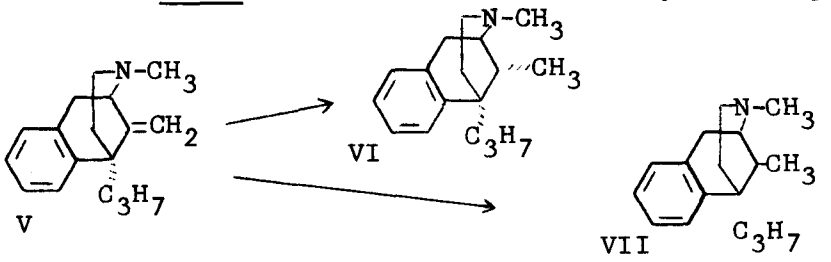
An interesting modification of the morphinan structure was described by Sawa and his colleagues.[3,4] They synthesized (-)-3-hydroxy-6,N-dimethyl-c-nor-morphinan III and the corresponding 6-oxo-isomorphan IV, the compounds with a 5-membered C-ring. The cis-fused compound was 19 x morphine and the trans-compound 12 x morphine in rats using the D'Amour-Smith test.

In the way of mechanisms, Way[5] and his co-workers have shown that heroin (H) is rapidly metabolized to monoacetylmorphine (MAM) and morphine (M). They postulate that the major effects of H are dependent on the formation of MAM. Unchanged H exerts only minor effects. H is biotransformed to MAM which then acts

as a carrier to facilitate the access of M to receptor sites in the C.N.S. Krivoy and his group have continued in their attempts to relate the development of tolerance to morphine with the formation of proteins or peptides in the C.N.S. which counteract the drug. Cohen et al.[6] report some intriguing experiments in which they are able to delay the development of tolerance to morphine by pretreating the animals with actinomycin D. Whether the inhibition of tolerance development is related to depression of RNA synthesis and consequent changes in protein synthesis remains to be shown as does the specific nature of the protein or peptide involved. The role of physical and psychological stress on analgesics was assessed by Frommel[7] who found that these stresses prolonged the action of morphine in guinea pigs.

B. Benzomorphans

May and his colleagues continued their elegant studies in this area. Chignell et al.[8] reported the preparation α-(cis) and β-(trans)-2-hydroxy-2',9-dimethyl-5-propyl-6,7-benzomorphan. Again, as has been previously reported for other members of this series, the trans-isomer is considerably more potent than the cis-isomer. The cis-compound, like other cis- and 5-monoalkyl derivatives in the benzomorphan series, has little or no ability to substitute for morphine in addicted monkeys. The trans-compound, however, does suppress withdrawal symptoms in addicted monkeys. Chignell and May[9] prepared the 9-methylenedesoxy compound (V) which they then reduced stereospecifically to the cis-(VI) and trans-isomers (VII). The methylene compound proved



to have about half the analgesic activity of morphine as did the trans-isomer. Jacobson and May[10] prepared a variety of 2'-nitro-, 2'-amino-, and 2'-halo-5,9-dialkyl-2-methyl-6,7-benzomorphans. Replacement of the phenolic hydroxyl by these other functional groups led to a diminution of analgesic potency and an increase in toxicity. As an interesting sidelight, it was reported that analgesic (Hot Plate) ED_{50} values in a new type of Caesarean derived general purpose mice were approximately 1/2 those previously obtained with other mice at this institution. It would appear that these healthier, faster growing mice are more sensitive to analgesics. Joshi et al.[11] prepared a homologous series of 5-alkyl-2'-hydroxy-6,7-benzomorphans, and have compared these with a series of cis-2-alkyl-2'-hydroxy-5,9-

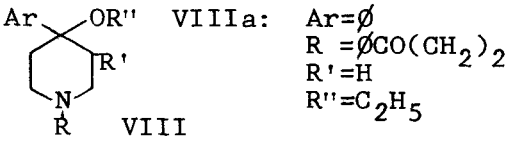
dimethyl-6,7-benzomorphans. In the 5-alkyl series the relative activity was Me<Et = Pr = Bu>Am>Hex. In the N-alkyl series they found Hex>Am>Me with the Et, Pr and Bu being inactive. It had earlier been reported[12] that the N-propyl compound is a potent analgesic antagonist.

Kametani and his colleagues[13,14] have reported an interesting modification of the benzomorphan nucleus. They prepared 1,2 3,4-tetrahydro-6H-1,5-(e)(1,4) diazocine and 1,2,3,4,5,6-hexahydro-2,6-methanobenzo (e)(1,4) diazocine, two azabenzomorphans. It will be interesting to ascertain to what extent the appropriate N-alkyl derivatives of these compounds exhibit analgesic activity.

C. Meperidine Derivatives

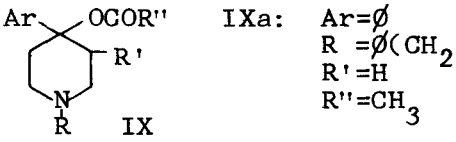
Despite the fact that over 4000 meperidine derivatives have been prepared, work in this area continues unabated. Hardy et al.[15] described several homologous series of nor-meperidines including N-alkyl, alkanol, alkyl ether, etc. where the N-side chain varied in length from 3-7 atoms. Their results indicate that the overall length of the side chain is quite important for analgesic activity. Peak activity consistently appeared when the side chain skeleton had an overall length of 7-9 A°. Their results indicate that within different series of compounds increasing the length of the N-side chain produces the same changes in analgesic potency. As they point out, this may be the result of equivalent effects on rates of transport to the active site or to similar changes in affinity for that site.

Casy and Armstrong[16] prepared a series of 4-alkoxy- and 4-acyloxy-4-arylpiperidines of the type VIII and IX for comparison



VIII

VIIIa: Ar=Ø
R =ØCO$(CH_2)_2$
R'=H
R''=C_2H_5

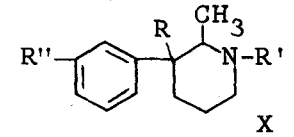


IX

IXa: Ar=Ø
R =Ø$(CH_2)_2$
R'=H
R''=CH_3

with the previously reported analgesic 4-ethoxy-4-(2-furyl)-3-methyl-1-phenethylpiperidine. They found the 4-phenyl-4-alkoxy derivatives to be generally more active than the corresponding 4-(2-furyl)-4-alkoxy compounds. The highest activity in the alkoxy series was found with the benzoylethyl derivative (VIIIa) (4 x meperidine) while among the acyloxy compounds the phenethyl derivative (IXa) was the most active (5.7 x meperidine).

Kugita and his colleagues[17] continuing their earlier work with 3-alkyl-3-phenyl-piperidines prepared a series of 3-alkyl-2-methyl-3-arylpiperidines of the general structure X. The N-phenacyl derivative (Xa) was found to be about as potent as



X

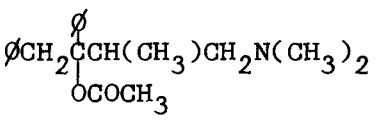
Xa: R=CH_3, R'=Ø$COCH_2$, R''=OH

Xb: R=CH_3, R'=CH_2=$CHCH_2$, R''=OH

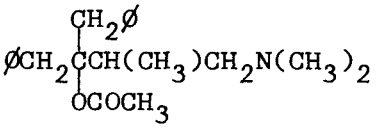
morphine as an analgesic in mice. The N-allyl derivative (Xb) proved to be an antagonist and was devoid of analgesic activity. This is of interest since this differs markedly from the meperidine series where the N-allyl compound is a straightforward analgesic. As was postulated by Archer and Harris,[18] antagonistic activity may be related to the phenethylamine fragment of molecule, a structural feature which Xb shares with the other narcotic antagonists and which is lacking in N-allylnormeperidine.

D. Miscellaneous

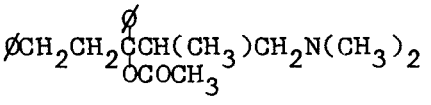
Casy and Meyers[19] have prepared a series of 1,2-diaryl-4-dimethylamino-3-methylbutan-2-ols. Substituents in the 1-phenyl ring reduced or abolished analgesic activity. Among the acyl esters, XI was 1.3 x meperidine. An additional methylene group



XI



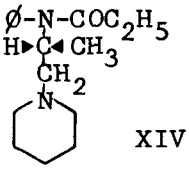
XII



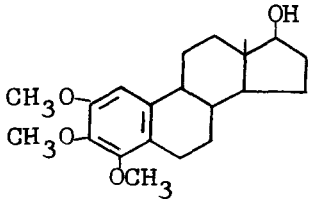
XIII

between C_2 and the C_2 phenyl (XII) did not affect activity. However, a methylene group between C_1 and the C_1 phenyl (XIII) essentially abolished activity. These changes in activity are related to the relatively rigid molecular structure of XI and XII as opposed to the less crowded more mobile structure (XIII).

Portoghese[20], in his continuing studies of the relationship of steric factors to biological activity has determined the absolute configuration of (-)-phenampromide to be (R)-N-(1-methyl-2-piperidinoethyl)propioanilide (XIV). (R)-Phenampromide, (S)-isomethadone, and (2S:3R)-propoxyphene are stereochemically related and have greater activity than their enantiomorphs. This



XIV



XV

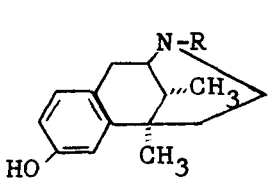
indicates a stereoselective attachment to a common receptor site. In a later paper[21] Portoghese has put forth a concept of the mode of interaction of narcotic analgesics with receptors. He postulates different modes of analgesic-receptor interactions depending on the type of series of agent. When parallel changes in activity are seen in a series of compounds with identical changes in the N-substituent, this would suggest similar binding

modes. Nonparallel relationships would then be indicative of dissimilar interactions. He has demonstrated quantitatively, in a series where the mode of binding is similar, that a linear free-energy relationship exists. He conceives of the receptor as having a charged anionic site as a common pivot point around which the analgesic may be more or less tightly bound depending upon its structural and conformational characteristics.

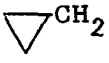
In the light of this, and our thinking about this field over the years, the note by Axelrod et al.[22] concerning the potent analgesic properties of a non-nitrogenous steroid XV is even more surprising. This compound was reported to be 40 x morphine in a modification of the rat tail-flick method when given by the intravenous route. In man, the compound administered intravenously, was stated to relieve postoperative and chronic cancer pain. Further details of this work are awaited with great interest.

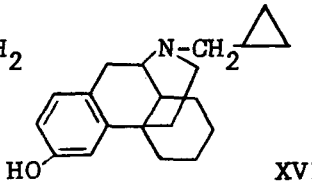
E. Narcotic Antagonists

It is, perhaps, among the group of compounds known as analgesic-antagonists that the greatest progress has been made in recent years in the field of analgetics. The topic has recently been critically reviewed by Archer and Harris.[18] The development of pentazocine (XVIa), cyclazocine (XVIb), and cyclorphan (XVII)



XVIa: R=$(CH_3)_2C=CHCH_2$

XVIb: R= 



XVII

has helped to change our concepts of drug development in this area. These agents antagonize the actions of morphine, are generally inactive or poorly active as analgesics in animals, yet are potent analgesics in man and have little or no morphine-like addiction potential.

1. Pentazocine

In the past year several studies[23-27] have confirmed the analgesic efficacy of pentazocine. The consensus of opinion is that 30-40 mg/70 kg is equivalent to 10 mg/70 kg of morphine. Side effects resembled those seen with morphine and the drug was remarkably free of nalorphine-like psychotomimetic properties. Like morphine, pentazocine produces some degree of respiratory depression.[28-30] Telford and Keats[28] have shown that this respiratory depression can readily be reversed by the analeptic, methylphenidate. In concomitant tilt table studies, Israel et al.[30] noted hypotension only rarely with pentazocine.

2. Cyclazocine

This compound is about 40 times more potent than morphine as an analgesic in man.[31] At equianalgesic doses it produces about the same degree respiratory depression as morphine but, like other analgesic antagonists, there is little increase in this

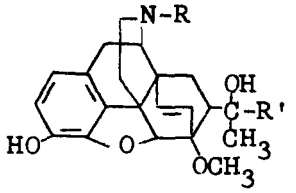
depression as the dose is increased. Cyclazocine produces a high enough incidence of nalorphine-like subjective effects to make it unacceptable as a useful analgesic. Martin and his group have reported[32] that tolerance develops to the subjective effects of the drug and that when patients are kept on high doses for prolonged periods and abruptly withdrawn, abstinence signs appear. These withdrawal symptoms are relatively mild and not typical of those seen with morphine. On the basis of these and other studies, Martin et al.[33] have proposed the use of cyclazocine in maintaining narcotic addicts free from narcotic abuse. Under the influence of cyclazocine the euphorogenic effects of narcotics are antagonized and the development of physical dependence is prevented. An outpatient clinic trial of this therapy is being carried out by Jaffe[34] and Freedman.[35] The initial reports are quite encouraging.

3. Cyclorphan

This drug is also a potent analgesic in man.[36] Like cyclazocine, it produces too high an incidence of nalorphine-like subjective effects to be a clinically useful analgesic.

4. Oripavine Derivatives

One of the most interesting findings in recent years has been the observation by Bentley and his colleagues[37] that ring expansion of morphine to the six-ring 6,14-endoethenotetrahydrothebaine or oripavine structure leads to agents of surpassing potency. Thus XVIIIa was found to be nearly 8,000 times more



XVIIIa: R=CH_3, R'=$(CH_3)_2CHCH_2CH_2$

XVIIIb: R=  CH_2, R'=CH_3

XVIIIc: R=  CH_2, R'=$(CH_3)_2CHCH_2CH_2$

potent than morphine.[38] These compounds have been used extensively to immobilize wild animals since their high potency makes darting quite feasible and their safety ratio in four-legged animals is high. Recently Bentley et al.[39] have described the N-cyclopropylmethyl derivatives in this series. This side chain has led to potent antagonists in the morphine, morphinan and benzomorphan series. In the 6,14-endoethenothebaine series when the C-alkanol side chain is isopropanol (XVIIIb) potent antagonism (35 x nalorphine) is produced. When R'=isoamyl (XVIIIc) the compound behaves more like a strong analgesic (250 - 1000 x morphine). There were, however, some qualitative differences between XVIIIc and morphine. Compound XVIIIb has been given to man[40] and has been found to produce a high incidence of nalorphine-like side effects.

5. Miscellaneous

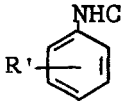
There has been some question as to whether the narcotic-antagonists are competing with the narcotics for a common receptor site in the C.N.S. Evidence favoring this view for nalorphine and levallorphan was put forth by Grumbach and Chernov[41] in a carefully controlled study. They carried out Gaddum Drug Ratio studies in rats with the two antagonists and a variety of analgesics. The ratios for four pairs of drugs were constant over a wide dose range indicating that the antagonism produced is a "surmountable" one and thus the drugs are competing for a common receptor site. The search for a specific animal analgesic test for this class of agent has continued. Ward et al.[42] compared nalorphine and pentazocine with morphine in a modification of the Randall-Sellitoe Test. Winter and Flataker[43] described a more complete study encompassing several antagonists. Nalorphine, pentazocine, cyclazocine, and N-cyclopropylmethylnormorphine raised the threshold of the yeast inflamed foot but had no effect on the control paw. (-)-3-Hydroxy-N-cyclopropylmethylnorisomorphinan was inactive. There was little relationship between the dose necessary to raise the threshold and the clinical efficacy of the drugs. Taber et al.[44] and Blumberg et al.[45] report the effectiveness of the antagonists in the mouse phenylquinone-writhing test. This has been expanded by Pearl and Harris.[46] To date, activity in this test gives the best correlation with clinical efficacy.

III. Weak Analgesics

Much of the laboratory work in this field has recently been reviewed[1] as has the clinical pharmacology.[47] Certain aspects of this drug class are covered in greater detail in the Chapter on Non-Steroidal Anti-Inflammatory Agents in the present volume.

A. New Agents

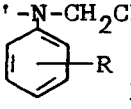
Takamura and his colleagues[48] have reported a series of aniline derivatives of the type XIX and XX. The compounds XIXa and b were the most potent analgesics. In an interesting study,



XIX

XIXa: R=N(CH3)2, R'=CH2=CHCH2O

XIXb: R=N(CH3)2, R'=p-Br

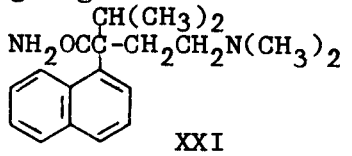


XX

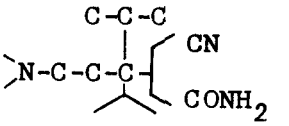
Ehrhart et al.[49] have prepared a series of β-hydroxybutyrates and α-dimethylamino-β-hydroxybutyrates of p-phenetidine and N-methyl-p-phenetidine. These compounds exhibited analgesic activity of about the same level as phenacetin but appeared to produce much less methemoglobin formation. This was attributed to a metabolic breakdown different from that of phenacetin. That is, there was no formation of p-phenetidine.

Casadio et al.[50] described a series of α,α-disubstituted-phenylacetonitriles, 1-naphthylacetonitriles, phenylacetamides, and 1-naphthylacetamides, many of which had analgesic and anti-

inflammatory properties. Most active were the 1-naphthylacetamides, in particular, naphthypramide XXI, which is currently undergoing clinical trial. Pala et al.[51] suggested, from struc-

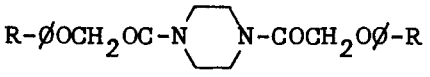


XXI

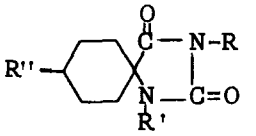


XXII

ture-activity studies of the whole series, that XXII represents the optimal structure for analgesic activity. Nishino and his group,[52] in a continuing study, prepared a series of 1,4-bis-(substituted phenoxyacetyl)piperazines XXIII, which showed analgesic activity. These compounds also appear to have C.N.S.

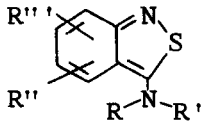


XXIII

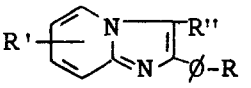


XXIV

depressant properties. Olin and Cashin[53] reported a series of cycloalkanespiro-5-hydantoins (XXIV), which were active in the mouse writhing test when R or R'' was a small aliphatic group or when R' was acetyl. These compounds also had anti-inflammatory properties. Meyer et al.[54] prepared a group of 3-amino-2,1-benzisothiazoles XXV, a large number of which showed anti-nociceptive activity in mice which was not correlated with their antibradykinin activity. Some resembled aminopyrine in being



XXV



XXVIa: R=p-SO_2CH_3, R'=R''=H

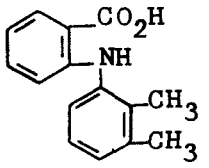
XXVIb: R=p-SO_2CH_3, R'=H, R''=NH_2

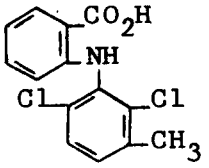
XXVIc: R=p-SO_2CH_3, R'=H
R''=$(CH_3)_2NCH_2$

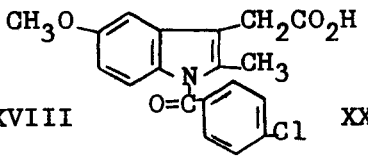
active in both tests. Almirante et al.[55] reported a series of imidazol-[1,2-a]pyridines of the general structure XXVI. The most interesting activity was found in the methylsulfonyl analogs (XXVIa, b, and c).

There have been reports[56] that the dimethylaminoethanol salt of 2-(4-biphenyl) butyric acid (namoxyrate) is an effective analgesic. The drug appears to be rapidly absorbed after oral administration[57] reaching a maximum plasma level at one hour. Namoxyrate and/or its metabolites are excreted slowly over several days. The animal pharmacology has not, as yet, been reported.

Mefenamic acid XXVII continues in active clinical research while another compound in this series XXVIII has been reported to be 150 x more potent than aspirin (1). Bissel[58] reported


XXVII


XXVIII


XXIX

mefenamic acid to be equivalent to codeine compound and superior to paracetamol in a double blind crossover study. Williams et al.[59] carried out a double blind evaluation of aspirin and XXVII for their ability to alter experimental ischemic arm pain. Both significantly increased pain threshold with no significant difference between medications. No placebo control was run.

Indomethacin XXIX is now being marketed in the U.S.A. An editorial in the British Medical Journal[60] sums up recent experience with this drug. In general, it has proven to be an effective mild analgesic, albeit with a relatively high incidence of side effects. These side effects, however, are considerably lower when the drug is given by capsule and the dose carefully adjusted. Indomethacin is particularly useful in the treatment of many of the rheumatic diseases.

B. Miscellaneous

Portoghese[61], continuing his studies of the relationship of structural conformation to activity, prepared the enantiomers of N-2-(p-methylbenzylmethylamino)propyl propioanilide and its N-propyl analog. The S-(-)-isomers had greater activity. There was a lesser potency ratio between the isomers of the N-methyl and the N-propyl compound. This was attributed to a diminution in the stereoselectivity of the analgesic receptors and might be related to differing modes of analgesic-receptor binding.

It has become apparent from the studies of Gilfoil[62] that inflammation and hyperesthesia are not a single phenomenon. Many agents produce swelling without attendant increase in pain sensitivity. A factor or factors which are released by certain agents which produce inflammation may be related to hyperesthesia. This factor is not serotonin or bradykinin. Winter and Flataker[43] using a variety of hyperesthesia-producing agents, have shown that indomethacin and the narcotic-antagonists can reduce hyperesthesia at doses which do not affect swelling and which do not affect the pain threshold of uninflamed tissue. This might reveal a central analgesic action in certain mild analgesics unrelated to their peripheral action. This work appears to be leading to a greater understanding of inflammatory pain and how the mild analgesics exert their effects.

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Chapter 5. Anorexigenic Agents

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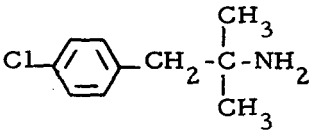
Introduction - Because of its efficacy and widespread clinical usage, amphetamine has been the standard anorexigenic drug for some time.[1, 2, 3] Efforts to reduce the central stimulant and cardiovascular effects of amphetamine have led to the development over the years of a number of new products which, hopefully, are free of undesirable side effects. Excellent summaries of these developments are available.[4, 5, 6, 7]

Along with these new drugs there has also developed a controversy. Are true anorexigenic and central stimulant effects separable?[6, 8] Can drug control of a "hunger center" in the lateral hypothalamus[9, 10, 11, 12] or of a "satiety center" in the ventromedial hypothalamus[13, 14, 15, 16] selectively overcome powerful psychological factors that may be associated with obesity?[6] If appetite and hunger are effectively drug-controlled in a completely selective manner, will the obese patient be miserable from deprivation without substitution?

Although investigations of anorexigenic agents in 1965 do not seem to have provided clear-cut answers to all of these questions, we may well be getting closer to the truth.

Newer Drugs - Both the laboratory and clinical investigation of amphetamine congeners have received considerable attention over the past several years. Of greatest interest seem to have been the chloro, trifluoromethyl and alkyl-substituted amphetamine analogs.

Chlorphentermine (p-chloro-α, α-dimethylphenethylamine)(I), which has been known for some time as an anorectic in animals[17] with little CNS stimulant effect,[18] was introduced into general clinical use in April, 1965. At effective anorexigenic dosages, chlorphentermine seems to produce little or no central stimulation[19, 20, 21] and has been reported to

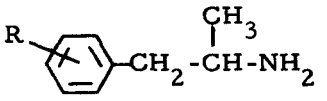


I

produce mild sedation in the form of drowsiness and relaxation.[22] Since chlorphentermine has been shown to inhibit lateral hypothalamic stimulus-induced eating in the satiated cat[23] as well as altering the evoked

potentials of hypothalamic "appetite centers", [23] answers to the questions mentioned earlier may come from observation of patient and physician acceptance of this new drug.

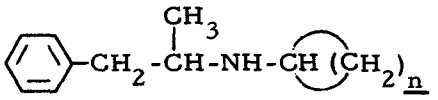
There are a number of reports on the investigation of ring-substituted amphetamine analogs (II). A series of 4-chloro, 2,4-dichloro and 3,4-dichloro compounds were only of modest interest.[24] An assortment of analogs monosubstituted at the 2, 3 and 4 position with methylthio, benzyl-



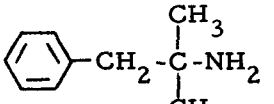
II

thio, methylsulfonyl, dimethylamino, isopropyl, aminosulfonyl, amino, benzoylamino, methylsulfonylamino and trifluoromethyl were examined for anorectic activity in the rat and dog. [25] Only the p-dimethylamino and m- and p-trifluoromethyl derivatives showed marked activity. The trifluoromethyl compounds appear to have little CNS stimulant effect and have been investigated extensively.[26, 27, 28, 29, 30, 31] The three isomeric monofluoro amphetamines are also reported to have substantial anorectic activity. [31]

Recent studies on N-substituted amphetamines have included the N-alicyclic compounds III where n was varied from 2 to 5. In this series,



III



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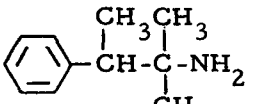
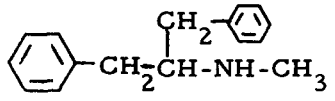


V

only the N-cyclopropyl compound was effective in depressing the food consumption of rats.[32] Phentermine IV [17] and 2-phenyl-3-methyl-3-aminobutane (V) [33] are recent examples of active amphetamine analogs substituted on the side chain. Multiple substitution such as with the N-methyl-α-benzyl-β-phenethylamine (VI), [34] the N-benzyl-p-chloro compound VII, [35] N-methyl-p-pentylamphetamine VIII, [36, 37] and N-ethyl-m-trifluoro derivative IX (fenfluramine) [38, 39, 40, 41, 42, 43] has given active compounds of interest. Compounds I, IV, V and IX have been compared with d-amphetamine as to potency and duration of the inhibition of food consumption in rats.[44] Both chlorphentermine I and fenfluramine IX were less potent at two hours but of about the same potency at twelve hours as d-amphetamine. In a three day, multiple-dosing experiment, they were clearly superior. [44] Metabolic studies on chlorphentermine [45] and fenfluramine [42]

showed a relatively slow catabolism which undoubtedly contributes to their longer duration of action.



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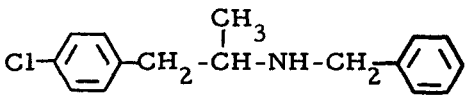
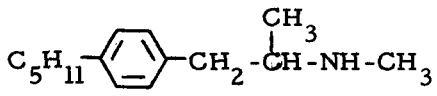
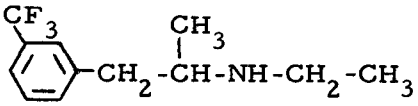


VII



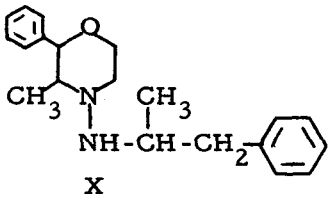
VIII



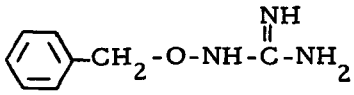
IX

Research activity with anorexigenic compounds more remotely related in structure to amphetamine continues. In most cases, one can detect a basic nitrogen atom approximately two carbons distant from a benzene ring.

A group of 4-aminomorpholines[46] related in structure to phenmetrazine were examined for their CNS effects.[47] The most interesting compound proved to be the N-(1-phenyl-2-propylamino) derivative X,



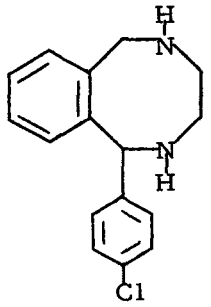
X



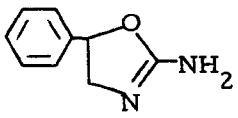
XI

which is an N-N combination of amphetamine and phenmetrazine. Compound X was shown to be an active anorexigenic agent with a behavioral profile different from that of amphetamine.[47] Aralkoxyguanidines have recently been reported to have anorexigenic activity.[48] The most active compound, benzyloxyguanidine (XI), is about 1/5th as active in inhibiting food consumption in dogs as d-amphetamine.

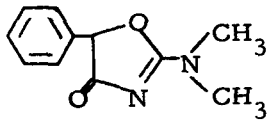
The discovery of anorexigenic activity for certain 2,5-benzodiazocines[49] provides another new class of potentially useful compounds. The pharmacological[50,51] reports on 1-(p-chlorophenyl)-1,2,3,4,5,6-hexahydro-2,5-benzodiazocine (XII) describe potency in the range of amphetamine with reduced central stimulant and cardiovascular actions. The potent anorexigen 2-amino-5-phenyl-2-oxazoline (XIII, aminorex), which was described several years ago along with a series of analogs,[52,53]



XII



XIII



XIV

has been reported to be an effective drug in obese diabetics.[54] In both rats and man, aminorex was shown to be rapidly metabolized and eliminated in the urine.[55] The structurally similar 2-dimethylamino-4-phenyl-2-oxazolin-4-one (XIV, thozalinone)[56] is also reported to be an active anorexigen with less acute toxicity and a longer duration of action than amphetamine when compared in mice.[57]

Large doses of α-methyl-m-tyrosine have been found to decrease the consumption of a sweetened milk solution by rats.[58] The long-lasting, dose-dependent effect is thought to be due to metabolites of α-methyl-m-tyrosine.[58] Anorexia has been attributed to imipramine.[59] Iproniazid has been shown to potentiate the anorexigenic effects of L-n-cocaine in rats.[60] The anorectic effect of L-n-cocaine is attributed to an amphetamine-like effect in the CNS rather than to its local anesthetic activity.[60]

Pharmacological Testing Methods - Classical procedures have measured drug effects on food consumption, weight gain or both.[61, 62, 63] Many refinements of these methods have been described.[64] One of the more interesting variations has been the use of obese animals, obtained either by destruction of the ventromedial nuclei[12, 65] or by chemical methods such as the aurothioglucose-obese mouse.[66, 67] The inhibition of drinking behavior has also been used as an index of anorexigenic activity. Effects on the controlled, short-term consumption of beef broth in rats have been suggested to more clearly reflect appetite suppression than feeding studies.[53] The use of sweetened milk solutions with rats also seems to give satisfactory test results.[58]

Perspectives - Most research in the anorexigenic area in the past has concentrated around compounds that are either chemically and/or biologically similar to amphetamine. As a result, many drugs are known which inhibit food consumption and concommitant weight increases in a manner similar to amphetamine. Available evidence suggests that several of these compounds exert their effect by a rather specific action in the hypothalamus without producing the central stimulant effects which are also associated with amphetamine. We now must wonder if such a drug alone will provide

adequate and reasonable therapy in obese humans. Perhaps a second pharmacological component will be necessary. Possibly this would be a "psychic stimulant", "psychic energizer" or anti-anxiety effect necessary to substitute for reduced food intake or relieve the underlying cause of overeating. It has been stated that over 90% of obesity in humans is psychogenic in origin.[6]

Although only centrally acting anorexigenic agents have been considered thus far in this review, the possibility should not be overlooked that metabolic, biochemical or hormonal factors might be drug controlled to combat obesity. Both d-amphetamine[68] as well as chlorphentermine, methylphenidate, pipradol, imipramine, desipramine and amitriptyline show a marked lipid mobilizing activity in rats as shown by increases in plasma free fatty acids.[69]

An anorexigenic and fat mobilizing substance (FMS) has been isolated from the urine of fasting rats.[70] It has been partially fractionated and is thought to be polypeptide in nature.[71, 72] The function and mechanism of action of FMS remain obscure.

Summary - Even though 1965 did not produce any obvious breakthroughs in the area of anorexigenic agents, there was one significant development. Chlorphentermine was introduced into widespread clinical usage in the United States during 1965. This drug appears to have a direct amphetamine-like anorectic effect in the hypothalamus with a much lower incidence of CNS stimulation than previous anorexients. Thus, an evaluation of the acceptance of chlorphentermine may provide an answer as to whether a "pure anorexigen" is desirable in the treatment of obesity.

If it turns out that some other central effect is a desirable attribute of an anorexigenic agent, then a superior drug may be available from a variety of active compounds that are in various stages of development. These vary from amphetamine analogs to somewhat different structures with a range of both quantitative and qualitative pharmacological differences from amphetamine.

Research effort continues to be concentrated on amphetamine-like anorexigenic action. The possibility of effective drug control of obesity by a completely different mechanism should not be overlooked.

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Section II - Pharmacodynamic Agents

Editor: S. Archer, Sterling-Winthrop Research Institute
Rensselaer, New York

Chapter 6. Antihypertensive Agents

E. C. Kornfeld, Eli Lilly and Company, Indianapolis, Ind.

Of the twenty-three new chemical entities introduced into medicine in the United States in 1965, none were concerned primarily with antihypertensive therapy.[1] The year was characterized, therefore, by the extension of established leads rather than by radical innovation. The following groups of drugs received attention:

Diuretics

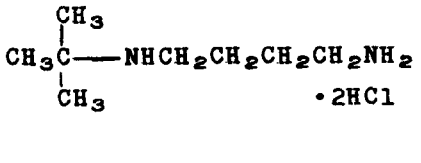
The oral diuretics of the thiazide or related class continued to be the one most important group of agents for use either alone or, more often, in combination with other hypotensive drugs. This area is reviewed in detail in Chapter 7. Use of the thiazides with potassium chloride to maintain potassium balance has led to small bowel ulceration problems;[2] however, administration of potassium in readily dispersible or slow-release forms appears to obviate this difficulty.[3]

Reserpine Analogs

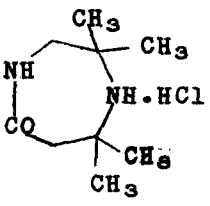
Application of the method developed by R. B. Woodward[4] for the total synthesis of reserpine has been extended to the preparation of unnatural analogs in France,[5] and other derivatives have been studied intensively in Czechoslovakia.[6] Reserpines with substituents in rings A, C, and E have been developed; however, it is uncertain as yet whether these offer a significant advantage over the natural alkaloids.

Ganglionic Blocking Drugs

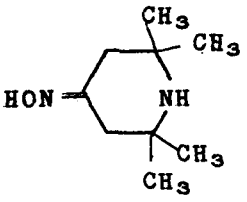
The application of the sterically hindered amine ganglionic blocking drugs, such as mecamylamine and pempidine, has diminished in favor of agents with fewer side effects. Nevertheless, work on a few related drugs has been published. Workers at Parke, Davis laboratories have reported on Dibutadiamin (I)[7] while pempidine derivatives such as II and III have been studied at Lakeside.[8]



I



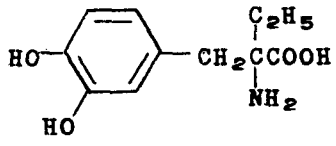
II



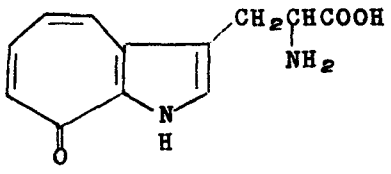
III

Amino Acid Enzyme Inhibitors

α-Methyl dihydroxyphenylalanine (α-methyl-DOPA) continued to find growing application as a moderately potent hypotensive agent. Although it is still the only drug of this class in wide use, a few related compounds have been studied. The α-ethyl-DOPA (IV) and the tropone derivative (V) appear to be

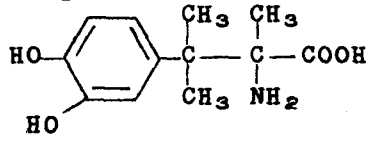


IV

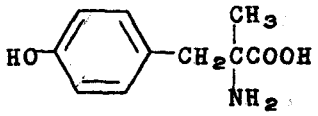


V

decarboxylase inhibitors,[9,10] while α,β,β-trimethyl-DOPA (VI) is reported to inhibit tryptophane hydroxylase.[11]



VI

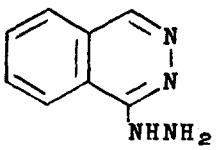


VII

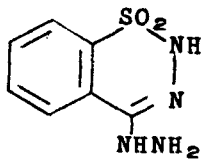
Still another member of this group, α-methyl-L-p-tyrosine (α-MPT) (VII), inhibits tyrosine hydroxylase. α-MPT has been shown to be effective in the treatment of hypertension caused by pheochromocytoma.[12]

Hydralizine Analogs

Although it is one of the oldest hypotensives, hydralizine (VIII) is still widely used, usually in combination with other agents. An analogous compound IX, reported from the Lakeside laboratories,[13] has been shown to lower blood pressure by decreasing peripheral resistance.



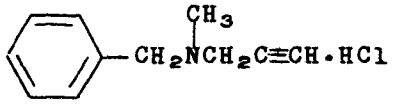
VIII



IX

M.A.O. Inhibitors

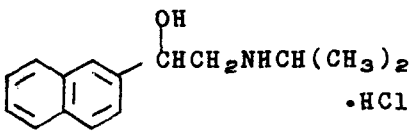
Interest in this group has waned because of problems associated with side effects and with hypertensive crises following concomitant ingestion of tyramine-containing foods.[14] Pargyline (X) is the best known of these agents.



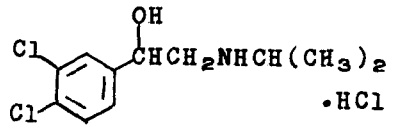
X

β-Receptor Blockers

Pronethalol (XI), which was developed following the lead

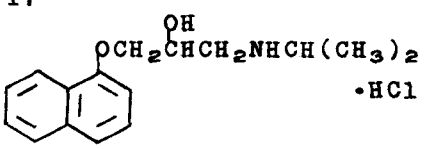


XI



XII

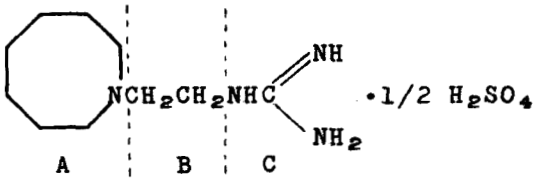
originally discovered in the drug D.C.I. (XII),[15] has been studied in the treatment of angina and arrhythmias. Sustained falls in blood pressure have been noted with this drug after oral administration for several months.[16] Pronethalol was subsequently shown to be carcinogenic in rodents, so trials have been shifted to propranolol (XIII), a related member of the series.[17]



XIII

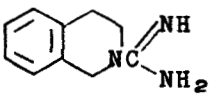
Guanethidine Analogs

By far the most intensive synthetic effort during the period has been directed toward the preparation of relatives of guanethidine (XIV). This drug has been described

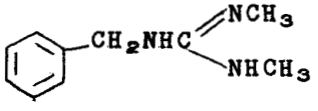


XIV

by I. H. Page as "the most satisfactory antihypertensive... to date"[18] for fairly severe hypertension. Consequently, the quest for even superior variants has been an active one. Guanethidine is composed of a heptamethylenimino portion (A) linked through a two-carbon chain (B) to a quanidine moiety (C). The following new analogs have been derived from guanethidine by variation of any or all of these three structural features. Clinical results are lacking with some of the new compounds; with others, it appears too early to assess the clinical utility relative to that of guanethidine. It appears likely that a number of the new drugs will be available. Debrisoquin (XV)[19] and Bethanidine (XVI)[20] have

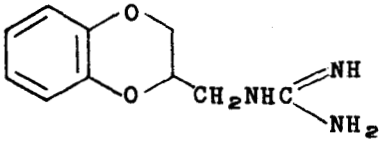


XV

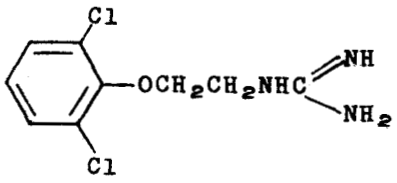


XVI

a shorter duration of action than guanethidine, a feature which may make dosage more flexible. Each is less likely to produce the diarrhea seen often with guanethidine. Envacar (Guanoxan) (XVII)[21] and Vatensol (XVIII)[22] have each been

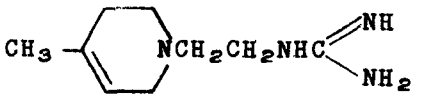


XVII

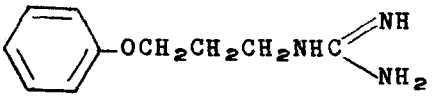


XVIII

found to be effective in certain guanethidine-resistant cases. Another clinically effective agent is the German drug, Guanacline (Leron) (XIX);[23] and a further analog with both hypotensive and antidepressant properties is Parke, Davis and Company's Guanoxyfen (XX).[24] The

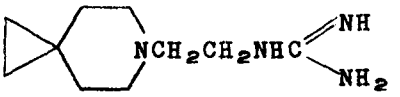


XIX

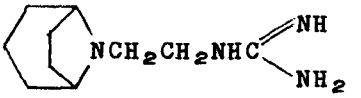


XX

guanethidine isomers XXI and XXII have also been prepared[25,26] and may offer some quantitative advantage over

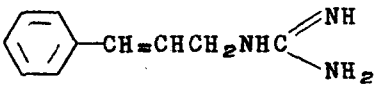


XXI



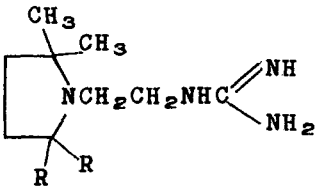
XXII

the parent drug. Of interest also from a stereochemical point of view is the observation that the olefinic analog XXIII is more active in the <u>cis</u> form than in the <u>trans</u>.[27]



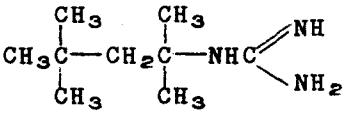
XXIII

The orally active <u>cis</u> form is more active and also of shorter duration than guanethidine. Finally, the following are listed to illustrate other modifications which have been reported:



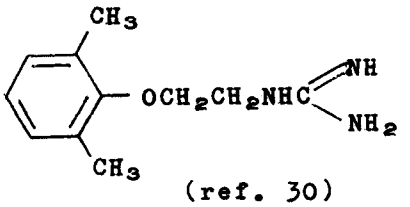
R = H, CH_3

(ref. 28)

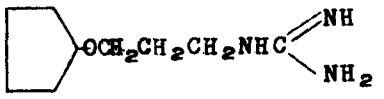


Guanoctine

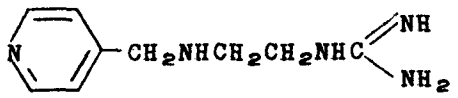
(ref. 29)



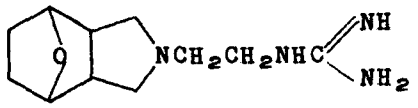
(ref. 30)



(ref. 24)



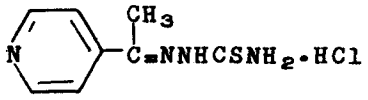
(ref. 31)



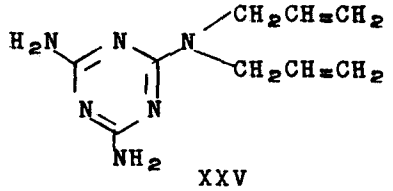
(ref. 32)

Miscellaneous Antihypertensives

The prostaglandins have shown some hypotensive properties,[33] but their role in this area is not yet fully elucidated. Among new synthetic types to show anti-hypertensive properties are the Bulgarian drug Depreton (XXIV),[34] which is claimed to be effective in human hypertension, and diallylmelamine (XXV),[35] which is hypotensive



XXIV



XXV

by virtue of direct action on vascular smooth muscle.

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Chapter 7. Diuretic Agents

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A perusal of the current literature, including the patent literature, leads one to believe that compounds with diuretic activity are most abundant. However, many of these reports are undocumented or the supporting biological data are meager. This review is restricted to selected reports that contain credible biological support or present chemical novelty. The literature coverage includes part of 1964 and extends through April 1966.

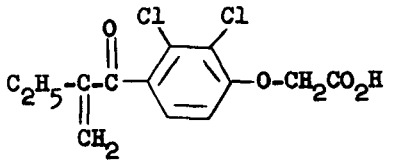
Organomercurials. The advent of newer diuretics has diminished the clinical importance of the organomercurial diuretics and few new compounds have been reported in recent years. Nevertheless, an active interest continues in the locus of action of this class of agents in the kidney nephron and the definition of the molecular events involved in the action of these substances on nephron function, particularly as it relates to sodium transport, potassium excretion and effects on uric acid and glucose metabolism.

Additional important evidence has been reported in support of the mercuric ion hypothesis[1] over the intact molecule hypothesis[2] for the action of mercurials. The mercuric ion theory[1] implies the presence in the kidney of mercuric ion during the period of active diuresis following organomercurial administration; solid evidence of this has been lacking. Using a novel isotope exchange diffusion analysis for Hg^{203}, evidence has been presented that the level of mercuric ion in the rat kidney correlated well with the onset and duration of diuresis after Hg^{203}-chlormerodrin. Mercuric ion was released also in kidney tissue of the rabbit and the chicken.[3] Similar results were obtained in the rat with p-chloromercuribenzoic acid,[4,5] an organomercurial that is not diuretic in the dog and does not release mercuric ion in a model *in vitro* system where other active mercurial diuretics readily yield mercuric ion.[4] However, this substance has been shown to be rapidly diuretic in the dog when administered by retrograde infusion into the renal tubular lumen.[6]

Important studies on the locus of action in the nephron[7] and the effect of the sodium reabsorption and oxygen consumption[8] of these mercurials have been reported.

Recently reported data support the concept that mercurial diuretics inhibit active sodium reabsorption *in vivo* by inhibition of the Na^+-K^+-activated component of the membrane ATPase system.[9] Thus, an enzyme system has been identified that can be correlated with active sodium and potassium transport and that is inhibited *in vitro* and *in vivo* by mercurial diuretics.

Acylphenoxyacetic Acids. Compounds of this type containing an α,β-unsaturated-acyl group were designed to react with functionally important sulfhydryl groups in a manner similar to that believed to occur with mercurial diuretics.[10] Biological data have now appeared indicating that such a reaction indeed does occur *in vivo* as well as *in vitro*. Ethacrynic acid (I) is



I

excreted, in part, in the dog as the cysteine adduct;[11] mercurials are known to be excreted as the cysteine conjugates.[12] The ethacrynic acid-cysteine adduct was shown to be an active diuretic in man.[13] In the dog, ethacrynic acid caused a decrease in protein-bound sulfhydryl groups in kidney cells as do the mercurials. It had no such effect in the rat, a species in which it is not diuretic.[14,15] The uptake of Hg^{203}-chlormerodrin by slices of renal cortex of the dog and the rat was reduced by ethacrynic acid.[16]

Using thirteen compounds of this class ranging from highly active to inactive in the dog diuretic assay, little correlation was found between diuretic activity and reactivity toward sulfhydryl (cysteine) in an in vitro model system. Better correlation was found with the ability to inhibit in vitro Na^+-K^+-(membrane)-ATPase from guinea pig kidney. However, the enzyme derived from the kidney of the dog and the rat was inhibited in vitro to approximately the same extent by ethacrynic acid.[17] Also, ATPase from the kidenys of rats that had received ethacrynic acid showed substantial inhibition without resulting in diuresis.[18]

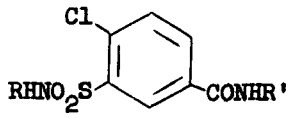
Substitution on the aromatic nucleus or on the unsaturated-acyl group markedly influences diuretic activity as measured in the dog. Compounds without nuclear substituents showed only weak activity. Substitution in the 2-position increased activity modestly while substitution in the 3-position or in the 2,3-positions, especially by chlorine or methyl, gave highly active compounds. Additional substitution in the 5 and/or 6 positions resulted in low activity. However, the 1,4-naphthalene analog was quite active. Substitution on the methylene of the unsaturated-acyl group reduced activity; the mono-methyl compounds showed reduced activity and the dimethyl compounds had only low activity. Saturation of the double bond of the acyl group yielded compounds with marginal activity.[10,17]

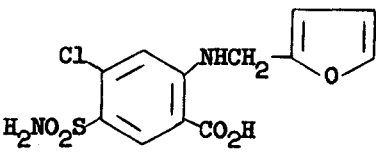
Detailed documentation of the pharmacology of ethacrynic acid and some of its congeners has appeared. In dogs, they caused a prompt and profound water diuresis and the excretion of nearly equivalent amounts of sodium and chloride when administered either parenterally or orally. There was some increase in potassium excretion, especially following intravenous administration. They have a steeper dose response curve and a substantially greater maximal saluretic effect than the thiazides, and at least as great an effect as the organomercurials. Results with combinations indicate a mechanism of action for ethacrynic acid different from that of the thiazides, acetazolamide and the mercurials. The ascending loop of Henle is a major site of action in the nephron.[11,19] Numerous reports on ethacrynic acid support its clinical efficacy.

Aromatic Sulfonamides. Interest continues in derivatives and structural variants of the m-benzenedisulfonamides. A number of 4-chloro-5-(substituted-amino)-m-benzenedisulfonamides and some 4-hydroxy analogs were effective

diuretics and the 4-chloro-5-(2-guanidinoethylamino) compound was weakly hypotensive.[20] Several 5- and 6-hydrazino derivatives were less effective in dogs and rats than dichlorphenamide.[21] However, the major recent interest in compounds of this class lies in analogs where one sulfamoyl group has been replaced by other electron attracting groups, such as, alkylsulfonyl, acyl or carboxyl and its functional derivatives. Diuretic activity in rats and mice has been reported for several 3-methylsulfonylbenzenesulfonamides.[22,23]

The carboxylic acid analogs have yielded highly active and useful drugs of current interest. Four compounds of this category have received considerable study. The chemistry and pharmacology of clopamide (II) has appeared earlier, but evidence of its clinical utility continues to appear.





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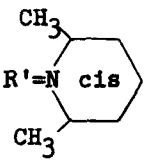


II, R=H; R'=N cis

III, R=H; R'=$N(CH_3)_2$

IV, R=R'=CH_3

The related acylhydrazine (III)(CI-546) was an effective diuretic, natriuretic and chloruretic in rats, dogs, monkeys and man. Results in the dog indicated that it exerts its effect on the renal tubule and that carbonic anhydrase inhibition becomes significant only at high doses. In man, kaluresis did not occur to any marked extent in the effective daily oral dose range of 20-320 mg. resulting in a favorable sodium-potassium excretion ratio.[24] Metabolism studies in several laboratory species, following oral administration, indicated rapid absorption and excretion principally in the urine with recoveries of 80-100% of the dose. The major urinary excretion products were the unchanged drug and a minor amount of the corresponding benzoic acid.[25] The related carboxamide derivative (IV)(CI-456) was similarly effective in the same species.[26] The methyl substitution on the sulfamoyl group of this compound is noteworthy. The fate of the methyl group is not reported but, from results with other methylated sulfonamides *in vivo*, demethylation to yield an unsubstituted sulfamoyl compound would be anticipated. The action of these compounds and the thiazides on active ion transport and smooth muscle contractility has been studied in relation to the hypotensive activity of the benzothiadiazines.[27]

Remarkable activity has been found in a series of 5-sulfamoylanthranilic acids appropriately substituted in the amino group.[28,29] Optimum activity was achieved when the amino substituent was arylmethyl or heterocyclic-methyl.

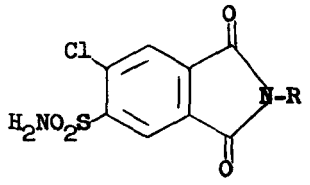
One member of this series, furosemide (V)(also designated, fursemide, frusemide) had strikingly different properties from the sulfonamides of all other types. However, the isomeric compounds, the 2-chloro-4-substituted-amino-5-sulfamoylbenzoic acids, were not saluretic.[28] The maximum naturesis, chloruresis and diuresis obtainable with furosemide, following oral or parenteral administration to man and to several species of animals, was several-fold greater than that produced by the most active thiazides.[30] Potassium excretion increased but, due to the massive sodium excretion, the sodium/potassium ratio is favorable. Renal studies indicated a site of action in the renal tubule different from the thiazides and mercurials; furosemide affects not only proximal and distal tubule sites but also the ascending limb of the loop of Henle.[31-34] These properties of furosemide are shared by ethacrynic acid, a non-sulfonamide, of entirely unrelated structure. Furthermore, interaction of furosemide with other diuretics in the dog indicated that the mechanism of furosemide is different from that of the thiazides and mercurials and is similar or identical to that of ethacrynic acid.[35]

Furosemide was excreted largely as unchanged drug in the urine[36] but 2-chloro-5-sulfamoylanthranilic acid, arising from loss of the furfuryl group, was identified as a metabolite.[37,38]

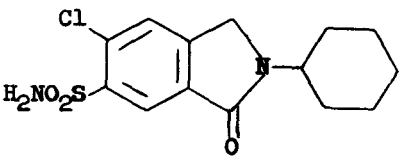
The clinical efficacy of this drug is well supported.[39,40] Furosemide is reported to produce the same side effects common to other sulfonamide diuretics, i.e. hypokalemia, hyperglycemia and hyperuricemia.[39,41]

Benzothiadiazines and Analogs. Many new publications on thiazide diuretics continue to appear, but no strikingly novel structural variants have been disclosed. Variations of the heterocyclic ring have given active compounds. The 3-aza analog of chlorothiazide, 6-chloro-2H-1,2,3,4-benzothiatriazine-7-sulfonamide 1,1-dioxide, showed activity comparable to hydrochlorothiazide in rats and rabbits.[42] 6-Chloro-thiochroman-7-sulfonamide 1,1-dioxide is reported to be active in rats.[43] 6-Chloro-3,4-dihydro-2H-1,2-benzothiazine-7-sulfonamide 1,1-dioxide (the isostere of hydrochlorothiazide in which the 4-NH is replaced by CH_2) was active in dogs.[44]

Detailed attention has been given to the isoindoline analogs, i.e. the phthalimides (VI) and the corresponding 1-oxoisoindolines. One compound of the latter series, chlorexolone (VII) was sufficiently active to justify clinical trial and has received further study.[45-47]



VI

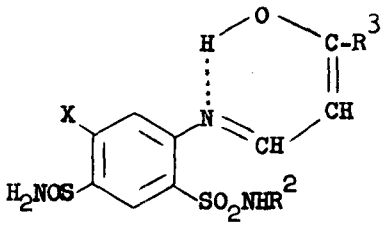


VII

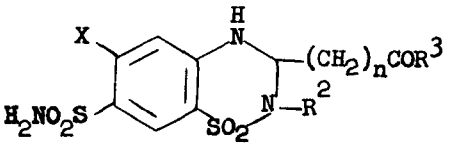
In each series, the nature of the substituent on the ring nitrogen had pronounced effects on activity as measured in the rat. Cycloalkyl groups were

most favorable in each series, and maximum activity was found with cyclohexyl or substituted-cyclohexyl although, in the phthalimide series, cyclopentyl, cycloheptyl and cyclo-octyl showed comparable activity. These derivatives were 3-6 times as active as chlorothiazide. Reduction of one carbonyl group to CHOH and to CH_2 (VII) increased activity some 10 and 50-fold respectively. Chlorexolone is 300-450 times as active as chlorothiazide in the rat and 50-fold in the dog. Conversion of the other carbonyl group to CH_2 to give the chlorexolone isomer abolished activity. The sodium, potassium and chloride excretion in this series resembled that of the thiazides.[45] Metabolism studies in rats and dogs showed chlorexolone to be excreted as monohydroxy derivatives of the cyclohexane ring.[48] Clinical results showed chlorexolone to resemble hydroflumethiazide in its action and to be equipotent.[46,47]

Of interest are the 3-(ketoalkyl) derivatives of the hydrothiazide series.[49,50] These are formed from the reaction of ketoaldehydes, $RCO(CH_2)_nCHO$, with the appropriate disulfamoyl aniline. With β-ketoaldehydes (n=1), intermediate anils were isolated when the ortho-sulfamoyl group carried at least one substituent. These were assigned the anil-enol structure (VIII). On further reaction, under acid conditions, the hydrothiazides (IX) were formed. Certain of these compounds (e.g. IX, $R^2=R^3=CH_3$, $X=CF_3$, n=1 or 2; VIII, R^2=allyl, $R^3=CH_3$ or C_6H_5, X=Cl) exhibited diuretic



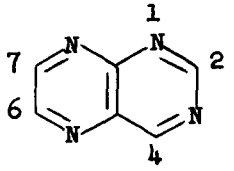
VIII



IX

activity in the dog comparable to trichlormethiazide. Particular interest lies in the hydrazones derived from IX and 1-hydrazinophthalazine (hydralazine), an antihypertensive agent. Some properties of two such compounds have been reported, EX-4877 ($R^2=CH_3$, $R^3=CH_3$, n=1, $X=CF_3$)[51] and EX-5004 (R^2=H, $R^3=CH_3$, n=1, X=Cl).[52,53] These hydrazones were not active diuretics but they showed a unique blocking action on the diuretic activity of the thiazides.[49] This blocking action of EX-4877 has been employed to analyze the mechanism of action of the thiazides.[51] EX-4877 did not alter the diuretic response to acetazolamide but it blocked the diuretic activity of hydrochlorothiazide. The activity of chlorothiazide was only partially blocked. Thus, it was concluded that EX-4877 inhibits the "B" (benzothiadiazine) effect of the thiazides, i.e. both hydrochlorothiazide and chlorothiazide, but leaves the carbonic anhydrase inhibitor "A" (acetazolamide) response of chlorothiazide unaffected.[51] EX-5004 was reported to be hypotensive in the rat and dog, probably by the lowering of peripheral resistance through a direct action on vascular smooth muscle.[53]

Pteridines (X). The diuretic properties of triamterene, 2,4,7-triamino-6-phenylpteridine, have been known for some time. Some structure-activity results have now been published. The nature of the substituents not only influence the potency of the compounds, but the qualitative characteristics of water and electrolyte excretion. In rats, 2,4-diamino-6,7-dimethylpteridine (SKF-371), like triamterene, produced saluresis and reduced potassium excretion.[54] The 2-anilino-4,7-diamino-6-phenyl derivative caused saluresis with kaluresis and no diuresis.[55]



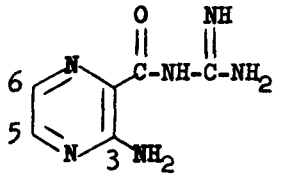
X

The 6-carbamoyl derivatives were generally more natriuretic than triamterene, especially in dogs. Although these derivatives produced little kaliuresis, they did not display the antikaliuretic effects exhibited by triamterene. Thus, 2-phenyl-4,7-diaminopteridine-6-carboxamide (SKF-6874) in the adrenalectomized rat receiving aldosterone produced natriuresis with no effect on potassium excretion. In contrast, triamterene increased sodium excretion and depressed potassium excretion.[54] Substitution on the carbamoyl nitrogen with or without simultaneous substitution on the 7-amino nitrogen can lead to increased activity in rats. In a series of N-R-2-phenyl-4-amino-7-NHR-6-pteridinecarboxamides, the most active compound was Wy-5256, where R=2-methoxyethyl. Replacing the 2-phenyl with p-methoxy- or p-chloro-phenyl, 2-thienyl, methylmercapto-, diethylaminoethylamino or hydrogen gave less active compounds as did replacing 7-NHR with hydroxyl.[56] In more detailed studies in rats and dogs, Wy-5256 was shown to have about the same oral diuretic activity as hydrochlorothiazide. About equal amounts of sodium and chloride were excreted along with some potassium and, when combined with hydrochlorothiazide, the effects of the two drugs were additive.[57]

Another substituted-amide, N-(2-morpholinoethyl)-2-phenyl-4,7-diaminopteridinecarboxamide (Wy-3654), in the dog produced marked sodium and chloride excretion with some potassium excretion. From studies on combinations of Wy-3654 with other diuretic agents, it has been concluded that the mode and/or site of action of Wy-3654 differs from that of hydrochlorothiazide, acetazolamide and ethacrynic acid but are similar to that of mercaptomerin.[58]

The metabolism of triamterene in humans results in five products in the urine: unchanged drug, 2,4,7-triamino-6-p-hydroxyphenylpteridine, the corresponding sulfuric acid ester, a N-glucuronide of triamterene and an unknown metabolite.[59] From the kidney tissue of triamterene-treated rats, a pteridine nucleotide has been isolated.[60] Clinical studies using a combination of triamterene and a thiazide indicated that normal serum potassium levels could be maintained without potassium supplements.[61]

Pyrazines. N-Amidino-3-aminopyrazinecarboxamides (XI) constitute a new class of diuretic agents.[62] They are unique both in structure and in their strong basic character. In normal rats, they produced marked diuresis while leaving unaffected or repressing potassium excretion. They antagonized the renal actions of exogenously administered aldosterone, DOCA or hydrocortisone in the adrenalectomized rat. Although the potency in dogs was lower, the relative activities of the various members of the series paralleled those in rats.[63]



XI

Halogen in the 6-position enhanced activity; the potency was in the order Cl > Br > I. The 6-chloro derivative (XII) was more potent than triamterene and considerably more active than spironolactone.[63] Introduction of alkyl, substituted-alkyl, alkylideneamino or aryl substituents on one or both of the terminal nitrogens of the guanidino moiety gave compounds that generally were less active than XII; however, derivatives of XII bearing N-benzyl, N-(2-hydroxyethyl) or N,N'-dimethyl substituents were equipotent with XII. Derivatives of XII having one acyl group on the 3-amino or on one or both of the terminal nitrogens of the guanidine moiety were considerably less active than XII.[62]

The introduction of a second amino group, at position-5, further improved activity. N-Amidino-3-amino-5-dimethylamino-6-chloropyrazinecarboxamide (XIII) was more active than XII in rats and dogs with a slower onset and a longer duration of action. When XIII was administered to rats, dogs or humans, it was demethylated stepwise to the 5-amino analog (XIV).[64] In rats, XIV was as natriuretic as hydrochlorothiazide with no kaliuretic effect. Small doses augmented the natriuresis produced by large doses of acetazolamide while decreasing potassium excretion. A synergistic increase in sodium excretion occurred when XIV and hydrochlorothiazide were coadministered while potassium excretion was considerably reduced.[65]

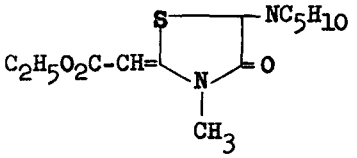
In dogs, XIV was qualitatively similar but less potent than in rats. Small doses of XIV in combination with either hydrochlorothiazide or ethacrynic acid produced enhanced natriuresis and depressed kaliuresis. Although the mode of action is different from spironolactone and organomercurials, stop-flow studies indicated that XIV inhibits distal potassium secretion and sodium reabsorption.[65]

In patients with cirrhosis and ascites, XIV, in doses of 8 to 30 mg./day, produced diuresis and saluresis with some bicarbonate excretion with reduced kaliuresis. In combination with hydrochlorothiazide or ethacrynic acid, XIV enhanced the excretion of sodium and decreased the excretion of potassium more effectively than spironolactone.[66,67]

s-Triazines. A recent critical study in rats has confirmed and extended earlier diuretic and natriuretic structure-activity relations in a large series of 2-mono-substituted-amino-4-amino-s-triazines.[55,68]

2-(p-Chlorophenylamino)-4-amino-s-triazine (Chlorazanil) has received considerable study in man. However, a more recent study indicated that the m-chloro isomer may be significantly more active in rats and dogs and in man.[69] In comparison with the present day potent diuretics, compounds of this class have little practical importance. The principle remaining interest lies in their modest effect on potassium excretion and in the modification of the action of several adrenalocorticoid steroids such as the antagonism of mineralocorticoids and of the deposition of hepatic glycogen induced by the glucocorticoids.[55] A detailed study has shown the influence on potassium excretion in the rat; at least, it is complex and strongly dose related. At non-toxic doses, most of the compounds can produce kaluresis.

Miscellaneous Heterocyclic Compounds. From a series of 2-methylene-4-thiazolidones,[70] the diuretic activity of one member, 2-ethoxycarbonylmethylene-3-methyl-5-piperidino-4-thiazolidone (XV), has been reported under the name Etozolin. It was effective in both the rat and dog.[71-76] In the



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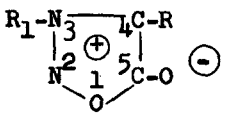
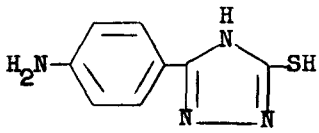


XVI



XVII

rat, it was reported to give a greater response than chlorothiazide, hydrochlorothiazide and acetazolamide.[74,75] In the dog, however, it was less active than hydrochlorothiazide and meralluride.[75] When given to dogs in clearance type experiments, it enhanced the maximal diureses of the mercurials and of chlorothiazide, indicating a probable mechanism different from these diuretics.[71] Results in man are not available.

Substituted sydnones (XVI) have been shown to increase the excretion of sodium and chloride in rats.[77,78] Of the three compounds reported in detail, the 3-butyl-4-ethyl derivative showed activity at lower doses than the 3-sec.-butyl compound and the 3-isopropyl-4-carboxylic acid; however, the activity was not great, and their potential usefulness is limited by their convulsive properties. A series of 3-(aminoalkyl)-sydnones were not active.[79]

In a series of thirty-nine s-triazoles, only the lead member, 5-(p-aminophenyl)-5-triazole-3-thiol (XVII) showed diuretic and natriuretic activity in rats at reasonable doses.[80]

The intense diuresis produced in the rat by a number of ethyleneimine derivatives was related to *in vivo* liberation of ethyleneimine. This marked polyuria was not accompanied by an increase in electrolyte excretion, hence the action bears no resemblance to that of the diuretics in clinical use.

An extrarenal mechanism may be involved, perhaps at the pituitary, since the polyuria was similar to that of diabetes insipidus.[81]

Aldosterone Antagonists. Although many 17-spirolactones of the steroid type have proven to be potent aldosterone antagonists in animals and in man, few new compounds of this type have been reported. However, the clinical utility of these compounds have stimulated the examination of other steroids for effects on electrolyte excretion in animals and in man. Oxygenated derivatives of progesterone[82-84] and testosterone[85] showed augmented activity. The acids corresponding to certain steroid spirolactones exhibited antagonistic activity comparable to the lactone.[86] Diuretic activity of some adrenocortical steroids in the rat have been compared. Cortisone, hydrocortisone and some of their 1,2-dehydro and fluorinated analogs have activity exceeding that of chlorothiazide.[87]

Compounds that are possibly non-steroid aldosterone antagonists have turned up in a series of 3-substituted-coumarins (e.g. 3-carboxyl, 3-acetyl, 3-glyoxyloyl). The activity is said to be confirmed in man.[88] An extensive review of aldosterone antagonists has appeared.[89]

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Chapter 8A. Angina Pectoris and Antianginal Agents

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During the last few years the symptom complex of angina pectoris has been the subject of an intense renewal of interest. Angina itself, by definition is pain, the easily described yet poorly understood clinical sign of a complex patho-physiological problem which occurs when the need for oxygen and possibly other nutrients by myocardial tissue exceeds the available supply. The need of the heart is governed by the work the heart must perform and the supply is dependent on an adequate coronary arterial system and flow of blood through this system. The oxygen saturation of blood, the state of the capillary bed, the ability of myocardial tissue to utilize oxygen, and the metabolic status of the heart are related factors. Atherosclerosis of the coronary vessels is the major etiologic factor yet it should be noted that angina pectoris can occasionally occur without evidence of coronary disease, as in severe anemia, congenital heart disease and advanced pulmonary disease. In contrast, some patients with evidence of coronary artery disease never experience angina.

Antianginal drugs have as their goal the alleviation, abolition or prevention of the symptom, angina pectoris, without adversely affecting cardiac performance. In addition, most drugs base their claim for antianginal efficacy on properties which hopefully will improve the basic coronary insufficiency. No compound available today alters the fundamental atherosclerotic process.

Nitroglycerin has been and still is the most important agent for aborting the acute attack of angina pectoris, though as we shall soon see, its efficacy has been challenged by some very thought-provoking experiments. In animals nitroglycerin acts as a pure dilator by increasing coronary flow, decreasing coronary arteriovenous difference and by effecting no significant change in cardiac oxygen consumption. The same was thought to be true for man. In 1959, Brachfeld[1] studied four normal and six mild cardiac patients before and after nitroglycerin. Following nitroglycerin, myocardial oxygen consumption increased as well as coronary flow. There was no change in cardiac work and a fall in myocardial efficiency was observed. Also noted were decreases in pulmonary artery, pulmonary wedge and right atrial pressures. At the same time, Gorlin[2] reported on seventeen patients, fourteen of whom had angina pectoris. Following nitroglycerin there was a decrease in cardiac output, blood pressure, and left ventricular work accompanied by falls in the systemic and pulmonary venous pressures. Coronary flow as measured by the nitrous oxide flow method was essentially unchanged and even decreased in some patients. Gorlin concluded that nitroglycerin acted by temporarily reducing the work of the heart rather than by relaxing the coronary vascular tree.

The time-honored conclusion that nitroglycerin increased coronary flow in anginal patients had been challenged using the technique of cardiac catheterization. Gorlin[2] confirmed his findings using I^{131} serum albumin to measure transcoronary circulation time with an external counting technique. Coronary circulation time was decreased in normal individuals after nitroglycerin but remained the same or increased in the abnormal group. In contrast, Johnson and Sevelius,[3] using radioactive iodopyrocet, demonstrated increases in myocardial blood flow in

arteriosclerotic patients following sublingual nitroglycerin and oral pentaerythritol tetranitrate. While the I^{131} substances seem to have fallen from favor, a variety of external counting techniques have been developed for the study of the coronary circulation. Rb^{86}, Rb^{84}, Na^{24}, Kr^{42}, Kr^{85} and Xe^{133}, among others, have been employed to measure myocardial blood flow.

Bing[4] has worked with a coincidence counting technique using Rb^{84}, a positron emitter, to determine coronary flow equivalent. With this method, nitroglycerin increased the clearance equivalent of Rb^{84} in patients without coronary heart disease and caused a decrease in clearance equivalent in patients with coronary disease. Ross[5] has measured human myocardial blood flow by determining precordial radioactivity following the injection of solutions of radioactive Kr^{85} or Xe^{133} directly into coronary arteries after coronary arteriography. The right and left coronary circulations could be studied separately and comparisons made with anatomical findings. No significant differences in flow at rest were found between normal and coronary artery disease patients. Bernstein[6] using Xe^{133} has studied the effect of nitroglycerin in thirty patients undergoing selective coronary arteriography for the evaluation of chest pain. Nitroglycerin (0.4 mg) sublingually caused a decrease in myocardial blood flow, left ventricular work and no significant decrease in coronary vascular resistance in patients with normal as well as diseased coronaries. Nitroglycerin (0.1 to 0.2 mg) injected directly into the coronary vessel caused an immediate though transient increase in myocardial blood flow and a decrease in coronary vascular resistance in the same patients. This blood level, attained by intracoronary administration, could not be achieved by the sublingual route using accepted dosages. However in attempting to explain the apparent conflict between arteriographic dilation caused by nitroglycerin and other nitrates[7] and the failure of these compounds to increase blood flow, Bernstein has presented a hypothesis based on a biphasic action. He suggests that during the first phase there is coronary vasodilation, a drop in coronary vascular resistance and increase in myocardial blood flow. During the second phase of the drug's action, the effects on the systemic circulation predominate and there is a decrease in arterial pressure and cardiac output. The decrease in coronary vascular resistance persists into the second phase, but because of the drop in arterial pressure, the myocardial blood flow falls below control levels.

Winbury[8] and Conn[9] have presented detailed reviews and critiques of the use of radioactive tracers to which the reader is referred. Ross[5] feels that the accurate location and discreet measurement of flow in unperfused areas of heart muscle which result from disease, may prove difficult using external counting techniques.

The advent of selective coronary arteriography[10] has made it possible to study the pathological anatomy of the diseased coronary artery. Sones[7] and Likoff[11] have demonstrated an increase in the calibre of the main coronary arteries and those branches large enough to be visualized following sublingual administration of nitroglycerin and other nitrates. Not only have presumably sclerotic vessels been seen to dilate, but also previously non-visualized collaterals were visualized following drug administration. However an increase in the calibre of a vessel, "vasodilation", does not imply an increase in myocardial blood flow. Winbury[8] has pointed out that smaller vessels which regulate flow cannot be visualized by the approach and questions whether or not the results obtained by

these techniques have any greater significance than those obtained by the measurement of blood flow. Their value in anatomic appraisal of the larger visible vessels cannot be challenged.

Nitroglycerin exerts pharmacological effects on both the venous and arterial circulation. Mason,[12] using normal patients, demonstrated that nitroglycerin dilated capacitance vessels of the forearm and caused significant pooling of blood. This finding represents additional evidence for the fall in cardiac output following nitroglycerin. Williams[13] demonstrated with cineradiograms a decrease in ventricular dimensions after nitroglycerin. These observations further suggest that nitroglycerin may relieve angina by reducing the work and oxygen requirements of the heart.

Cohen[14] has attempted to correlate in sixty patients the findings of selective coronary cinearteriography with clinical history, physical examination, electrocardiogram, Kr^{85} coronary blood flow studies, and myocardial oxygen and lactate extraction at rest and during stress. Some predictions could be made from the patient's history which were not previously possible. Disease of a single coronary vessel usually indicated pain less than one year's duration. Increasing duration in years revealed more extensive pathology. Pain limited to the chest usually indicated milder anatomic pathology than when the patient had pain in two or more sites or experienced prandial or nocturnal angina. Disease of three vessels was more common in this group. Seventy percent of the resting electrocardiograms were abnormal and two-thirds of the remainder were abnormal with exercise. The measurement of coronary flow at rest or after stress could not distinguish patients with coronary disease from those without, and no consistent correlation could be made with anatomic pathology. Myocardial lactate production or decreased extraction of lactate by the myocardium could be correlated with the severity of disease.

Raab[15] feels that interest in the metabolic and neuro-regulatory factors affecting the myocardium has been lagging due to a "one-sided preoccupation" with vascular anatomic factors. According to Raab, data on total coronary flow, arteriography and coronary sinus oxygen saturation do not locate circumscribed areas of anoxia or define the causative mechanism. In addition to the atherosclerotic process he feels that environmental, emotional and nicotine-induced over-stimulation of the sympatho-adrenal system and the deterioration of counter-regulatory mechanisms secondary to physical inactivity should receive more attention. Since catecholamines augment the uptake and consumption of oxygen far beyond energy requirements, factors such as stress, smoking, emotion and unusual physical exercise must be controlled by measures which can cushion this sympathetic activity. Emotional relaxation, nicotine abstinence and systematic physical training are recommended.

Thus far we have limited our review to a few of the current techniques used to study the coronary circulation and in so doing have shown some of the controversy surrounding the mechanism of action of nitroglycerin. The problem of angina pectoris and its relief is equally complex. Angina cannot be shown by the arteriogram or measured by coronary sinus oxygen or lactate extraction studies, or identified by the varied techniques used to calculate coronary flow. Vasodilation is not synonymous with increased coronary blood flow and neither expression means relief of angina. Several factors, the exact inter-relationship of which are not understood are probably etiologic, and relief of angina

can probably be attributed to several mechanisms which are also not fully understood as yet.

Sublingual nitroglycerin has long been considered the most useful agent for the relief of acute anginal attacks and for the prevention of an attack if taken prior to a stressful situation known to precipitate angina. While evidence has been mainly subjective, various exercise tests have been developed to achieve objectivity. Nitroglycerin and other compounds are studied for their effect on prevention, delay or decrease of anginal pain associated with a standard amount of exercise. An evaluation of simultaneous or post-exercise electrocardiographic changes may be included in the evaluation. Bernstein[16] has criticized these approaches because the patients studied are often limited to those whose anginal attacks are reproducible after a given amount of exercise or to those with reproducible electrocardiographic abnormalities. These patients represent but a small fraction of the total group of patients with angina pectoris.

Sandler,[17] using a double-blind technique has reported that nitroglycerin does not alter the duration of an established anginal attack when compared to placebo, but that the drug increased work performance and shortened the duration of ensuing angina when administered prophylactically three minutes before exercise. Russek[18] corroborated this later phase of Sandler's experiment giving nitroglycerin five minutes prior to exercise. Fisch[19] has been unable to demonstrate any effect of nitroglycerin on the duration of the actual attack, and in addition, found the drug no better than placebo when given three, five and ten minutes before exercise. Because of the difficulty in matching nitroglycerin with a perfect placebo Fisch feels that the responses of patients in the previously mentioned studies may have been influenced by drug recognition. This controversy involving nitroglycerin accentuates the host of difficulties inherent in the evaluation of drug efficacy in angina pectoris. If the attacks are of very short duration, for example, under ninety seconds, it is conceivable that the effects of nitroglycerin might not have time to be distinguished from those of placebo. It should also be pointed out that Fisch's patients were carefully picked for reproducibility of symptoms and are not necessarily representative of the majority of patients with angina pectoris. While angina is frequently related to effort, it also occurs at rest, during or after eating and at the time of emotional stress.

Nitroglycerin, despite controversy, remains the drug of choice for immediate relief from an acute anginal attack and for very short-term prophylaxis. Amyl nitrite though faster in onset of action, has a shorter duration of action and must be inhaled. Isosorbide dinitrate and erythrol tetranitrate are used sublingually, and by this route have been shown to afford clinical protection in anginal patients for periods up to two hours. These agents have little or no value in aborting the acute anginal episode. Newer sublingual nitrates, such as clonitrate[20] and stereo-isomers of pentanitrates[21] do not seem to possess any advantages over nitroglycerin.

Several long-acting nitrates are available for long-term prophylaxis of anginal attacks. This concept of therapy is far different from treatment of acute attacks or the relatively short prophylaxis mentioned previously in relation to the sublingual agents. The efficacy of the organic nitrates available for oral administration such as pentaerythritol tetranitrate, erythrol tetranitrate,

isosorbide dinitrate and trolnitrate phosphate must be established by long term investigation. As a class they seem to have pharmacological activity similar to nitroglycerin[22-24] but the results of long term treatment are difficult to evaluate in terms of their effect on anginal pain. Investigations conducted without placebo control are generally enthusiastic, while those employing double-blind methods have frequently not shown a statistical difference from placebo. The stresses of every day living involve a myriad of difficult-to-measure variables which influence the number and severity of anginal attacks, and therefore, a high degree of placebo responses can be expected. Bernstein[16] in considering this problem, suggests that treatment periods continue at least one year for each drug under evaluation. Unfortunately, longer studies of this type are extremely difficult to undertake from a practical standpoint.

Dipyridamole, a relatively new entry in the antianginal field, and quite unrelated to the nitrates, has a chemical nucleus of pyrimido-pyrimidine rings. When administered intravenously or orally to laboratory animals, the drug increases coronary blood flow and coronary sinus oxygen content. Normal individuals, as well as patients with impaired coronary circulation, have shown similar increases in these parameters following intravenous administration.[25] Laboratory studies with heart preparations have shown dipyridamole to protect against disturbances in function and fall in high energy phosphate levels induced by hypoxia.[26] Heart mitochondria of animals pretreated with the drug and exposed to hypoxia demonstrate preservation of anatomic structure.[27] Vineberg[28] employed long-term oral administration of dipyridamole in animals with experimental coronary occlusion, resulting in enhanced survival and increased coronary collateral circulation as compared to untreated controls. Bunag[29] has shown that dipyridamole, in the presence of intact red blood cells, prevents the deamination of exogenous adenosine suggesting this mechanism may account for effects on the coronary blood flow. Recently Emmons[30] and co-workers demonstrated a favorable effect by dipyridamole on human platelet aggregation.

Dipyridamole is not intended for the relief of an acute attack of angina pectoris but for long-term administration. In some instances several months of continuous therapy may be required to achieve a satisfactory response. Clinical observations without placebo controls in long-term treatment of angina pectoris have been quite enthusiastic and investigations have run as long as 24 months.[31] The results of studies using placebo controls and the double blind technique have been equivocal.

Chromonar hydrochloride[32] and phenylamine,[33] not yet available commercially in the United States, are agents that increase coronary blood flow. In addition, phenylamine is reported to have sympatholytic, adrenolytic and central sedative actions. Clinical reports from abroad have been, for the most part, encouraging.

The monoamine oxidase inhibitors have been used in the therapy of angina pectoris as well as in hypertension and certain psychiatric disorders. While experimental evidence for this effect on angina may be scant, several theories regarding mechanisms of action have been proposed.[34] These include central nervous system stimulation, mood elevation, central analgesic effect, increase in coronary flow (though this seems unlikely in man), a blocking effect on pain transmission, and decreased oxygen requirement of the tissues. However, there is no clear pharmacological basis for beneficial effects of these compounds on the coronary circulation or the myocardium.

According to a recent review[35] most clinical studies indicate that the monoamine oxidase inhibitors cause a subjective increase in exercise tolerance and patients can do more work during standardized exercise tests than during control periods. Nevertheless, ischemic ECG changes are not altered or delayed and may be more severe. Controlled and uncontrolled studies, while not unanimous, do seem to suggest some effect on anginal pain or the patient's reaction to it. This group of drugs can produce serious toxic reactions which have limited their use, by and large, to cases resistant to other forms of therapy.

The use of beta-adrenergic blocking agents in the therapy of angina pectoris has been the subject of recent interest. Since the catecholamines increase oxygen utilization by the heart as previously discussed,[15] compounds that may block this effect present an interesting approach to the problem. Pronethalol has been studied fairly extensively abroad. The drug reduces heart rate at rest and after exercise. While there appears to be a normal exertional increase in stroke volume, overall cardiac output after exercise may be reduced.[36,37] The drug should not be used in patients with cardiac failure or impending failure because of its myocardial depressant action. There were several favorable reports on the use of this agent in angina pectoris. More extensive use has been partly limited by frequent CNS side effects and by a report of its tumor-producing activity in mice.[38] Propanalol has a similar mode of action, and has been reported to have a therapeutic effect ten times greater than pronethalol without carcenogenic potential. Initial impressions of this agent have been generally favorable.[39-41]

No attempt has been made to consider all possible approaches to the problem. The use of sedatives and tranquilizers in angina pectoris can be quite useful for those individuals in whom anxiety, excitement and tension are precipitating factors. Alcohol in moderation, should not be overlooked in this regard. Papaverine, xanthines and anticoagulants have their advocates. Treatment of coexisting cardiac decompensation, arrythmias and hypertension benefits the anginal patient, as does reduction of weight and correction of anemia. Chemical or surgical thyroidectomy has been successful in some severe cases. Interest in surgical revasculization seems to be gaining some momentum. Though many if not all approaches are probably beneficial to certain individual patients or groups of patients, none as yet represent the ideal. None cure or halt the atherosclerotic process. The search must continue.

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Chapter 8 B. Antiarrhythmics

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From the therapeutic approach, despite numerous investigations, the degree of advance in the area of antiarrhythmic therapy has not been as marked as one might desire. Every year many new drugs are reported, but few ever reach clinical evaluation and none have thus far surpassed the traditional agents. A systematic synthesis of antiarrhythmic and antifibrillatory compounds is rendered difficult by the fact that most antiarrhythmic agents are quite different in chemical structure. A number of substances with entirely different chemical structures are known to possess antiarrhythmic activity and it is extremely difficult to attribute this action to a basic structure common to all agents in this field. Diverse drugs may abolish or prevent arrhythmias by influencing different cardiac parameters (e.g., refractoriness, excitability, rhythmicity and conductivity). Cardiac stimulants or depressants, sympathomimetics, sympatholytics, agents enhancing catecholamine depletion or storage, cholinergics, anticholinergics, local anesthetics, antihistaminics, autonomic agents and central neural depressants - all have offered numerous pathways for pharmacological investigation. Thus a clear demonstration of structure-activity-relationships is relatively impossible and meaningless.

The basis for this relative lack of success may be in the anatomical and physiological nature of the heart itself, which, from the standpoint of arrhythmias, can really be considered to possess five distinct structures. The specialized conduction system, which allows the heart to contract in a synchronous manner, consists of the sino-atrial node, the atrioventricular node, and the Bundle of His and diffuse Purkinje fiber system. In addition, the auricular and ventricular musculature also conducts impulses, and may, under certain conditions, initiate impulse formation resulting in ectopic beats. Disturbances in either the conduction system or musculature, can lead to a disorganized beat and inefficient cardiac contraction which in turn may diminish cardiac output. Such disturbances in impulse formation and/or conduction are referred to as cardiac arrhythmias, though a preferable term might be dysrhythmias.

Since most serious arrhythmias result in a faster than normal ventricular rate, it is not surprising that the two principal agents, quinidine and procaine amide have, as a common property, the ability to depress impulse formation and, therefore, rate. As mentioned, there are many chemical substances that can accomplish the same end, but quinidine and procaine amide also possess pharmacological specificity for cardiac tissue that other agents often lack. Cardiac glycosides (alone or in combination), although usually used to improve the contractile force of the heart, are often employed to depress specific areas of the arrhythmic heart with the hope of restoring a more normal conduction time and efficient ventricular rate.

The complex nature of the problem has of necessity forced pharmacologists to resort to a number of different laboratory models in order to screen potential antiarrhythmic compounds, for no one model is sufficiently useful by itself. The pharmacologist must test for action upon rate production, excitability and conduction time. Derangements of impulse formation due to chemical

agents (e.g. aconitine, cardiac glycosides, acetylcholine, catecholamines, etc.) or physical events (electrically or surgically induced arrhythmias) must be measured, and the degree of protection afforded by a test substance compared to a standard agent, usually quinidine. And, he must be ever mindful of the toxic properties of a new compound. These difficult laboratory limitations have to date, yielded no chemical agent possessing greater therapautic efficacy than the three time-tested stalwarts, quinidine, procaine amide and cardiac glycosidides. What is equally disturbing is the fact that the original observations of the antiarrhythmic properties of these substances resulted, not from laboratory study, but rather from astute clinical observation.

The presence of acetylcholine and norepinephrine in proper ratio in the functional heart is important in the maintenance of cardiac rhythmicity.[1] The ideal antiarrhythmic should therefore support a balanced ratio between cardiac norepinephrine and acetylcholine, yet at the same time it should ensure a diminution of myocardial excitability to circulating catecholamines. In addition, the ideal agent should depress the intrinsic rhythmicity of the conducting pathways, and regulate the flux of intracellular sodium and potassium, thereby maintaining ionic balance.

It seems obvious from the outset that no one drug will be found to possess all the properties inherent in the definition of the ideal antiarrhythmic. More likely, the ideal agent becomes many drugs capable of interfering with, or reversing, the numerous abnormal physiologic patterns of the heart.

1. Beta Adrenergic Blockers. With the availability of beta adrenergic blocking agents, pharmacologists have been handed a most important research tool. The agents of greatest current interest are dichloroisoproterenol (DCI), MJ 1999, pronethalol, propranolol, and N-isopropyl-p-Nitrophenylethanolamine (INPEA). All have been shown to protect against the initiation of various laboratory induced arrhythmias and to terminate some existing arrhythmias[2]. Pronethalol and DCI have been reported to afford protection against ouabain-induced fibrillation in guinea pigs[3] and to reduce arrhythmias resulting from a combination of ouabain plus electrical stimulation[4]. Doubt has been cast, however, as to whether the antiarrhythmic properties of these agents are solely attributable to their beta adrenergic blocking action.[5,6] Recent evidence obtained by comparing the beta adrenergic blocking and antiarrhythmic activity of the dextro-isomer and the racemic mixture of propranolol has resulted in the current view that antiarrhythmic activity is in part due to a non-specific (depressant) "quinidine-like" action, as well as beta blocking activity.[7] This conclusion is based in part on the fact that the racemic mixture of propranolol reversed the arrhythmias resulting from coronary ligation plus epinephrine, but the d-isomer did not; yet both agents reversed ouabain-induced arrhythmias and neither was effective against ventricular arrhythmias due to coronary ligation alone. Since the d-isomer possesses only 1/50 the beta adrenergic blocking action of the racemic mixture, it appears that in those arrhythmias associated with catecholamine release, beta blockade is of importance.

A number of studies have been performed comparing the antiarrhythmic activities of these agents with quinidine.[8,9] The results obtained appear to depend on the laboratory models employed, but, in general, propranolol is more potent than pronethalol insofar as beta adrenergic blocking and antiarrhythmic activity are concerned. Several British clinicians have also reported success in the treatment of ventricular arrhythmias with propranolol.[10,11]

Pronethalol is somewhat different than its newest derivative, propranolol, in that it possesses both sympathomimetic activity and an antivagal action, both of which result in increased heart rate. Moreover, pronethalol lengthenes the refractory period, diminishes excitability and is a potent local anesthetic equal to procaine.[12,13]

One of the newer agents, INPEA, was compared to pronethalol for antiarrhythmic activity.[14,15] Both agents antagonized catecholamine-induced arrhythmias (epinephrine and methylchloroform plus epinephrine), but were ineffective against the arrhythmias resulting from coronary artery ligation. Furthermore, only pronethalol was effective in reversing ouabain-induced ventricular tachycardia, indicating once again, that pronethalol also possesses a nonspecific depressent action, similar to quinidine.

MJ 1999 is a beta adrenergic blocker which also has the ability to antagonize cardiac irregularities produced by (large doses of) catecholamines, but affords no protection against the arrhythmias resulting from ouabain or coronary ligation.[16] Insofar as protective potency against arrhythmias is concerned it appears that propranolol > pronethalol > MJ 1999.

2. Catecholamine Depletors. It follows that since beta adrenergic blocking agents are able to antagonize certain types of arrhythmias that catecholamine-depletion might also afford protection against some arrhythmias. In 1963 Roberts et al.[17] reported that following reserpinization the ability of digitalis to induce ventricular arrhythmias was diminished. Moreover, βTM 10, an agent which prevents the release of catecholamines, markedly reduced the response of the ventricular pacemaker to digitalis. The ability of reserpine to antagonize arrhythmias has been confirmed by several workers [18,19], but it should be noted that the degree of success is much higher if an adrenalectomized preparation is employed so as to reduce the circulating catecholamines. In addition, guanethidine and bretylium have been reported to antagonize the arrhythmias produced by acetylcholine and electrical stimulation.[20,21]

3. Sympathomimetic and Pressor Agents. Certain types of arrhythmias (especially supraventricular tachycardias) may be converted to normal sinus rhythm by raising the blood pressure, thereby eliciting reflex vagal stimulation. Ellis [22] has reported that methoxamine and some closely related substituted phenylisopropylamines can effectively convert ventricular tachycardias to sinus rhythm. Special mention was made of α-methyl-β-hydroxy-β-(2,5 diethoxyphenyl)-N-isopropyl ethylamine, which was found to be effective in restoring sinus rhythm under a number of experimentally produced ventricular tachycardias (e.g. ouabain, epinephrine with or without hydrocarbon anesthesia, DMPP and coronary artery ligation). Similarly, isopropyl methoxamine has been found to be effective in converting digitalis-induced ventricular tachycardia to sinus rhythm, though the duration was rather transient.[23] In their review on the use of vasopressor drugs in the therapy of cardiac arrhythmias, Corday and co-workers[24] note that in addition

to methoxamine, such agents as norepinephrine, mephentermine, metaraminol, phenylephrine and angiotensin have been used successfully. However, such therapy is only useful when the arrhythmia is associated with hypotension. A possible exception lies in the usage of angiotensin which has been found to protect against a variety of experimentally induced arrhythmias both in vitro and in vivo.[25,26] Accordingly, these investigators also ascribed a "quinidine-like" action to angiotensin.

In the presence of heart block, ventricular tachycardia and quinidine intoxication, isoproterenol has been used successfully.[26] The mechanism involved is apparently based on the fact that isoproterenol accelerates the basic idioventricular pacemaker thereby suppressing ectopic ventricular activity. The increase in heart rate, coronary flow and cardiac output may also be responsible for terminating arrhythmias. Of course, driving the heart at a more rapid rate could achieve the same result as drug therapy. The inherent danger in the use of isoproterenol is that it is almost a pure beta adrenergic stimulator and may precipitate ventricular tachycardia or fibrillation.[27]

4. Local Anesthetics. Since the observation that procaine was effective in suppressing arrhythmias, and the subsequent development of procaine amide, numerous local anesthetics have been employed in antiarrhythmic drug therapy. Lidocaine, a synthetic local anesthetic, has recently been reported to be of value in the treatment of cardiac arrhythmias.[25] The mechanism of action appears to be similar to that of procaine. The conduction time is slowed, excitability depressed and the refractory period prolonged. In therapeutic doses myocardial contractility does not appear to be depressed nor is the blood pressure depressed. It has a brief duration, is relatively safe and may be given i.v., all of which may make it useful in arrhythmias which sometimes occur during cardiac surgery.

Pruss and Hidalgo[29] have reported on a new substance Levoxadrol®, which is a local anesthetic and a ganglionic blocking agent. The authors claim that it is slightly less potent than quinidine but more potent than either procaine amide or lidocaine in reversing atrial fibrillation caused by the topical application of acetylcholine. Moreover, it is very effective in restoring sinus rhythm in dogs with coronary artery ligation ventricular arrhythmias.

5. Antihistaminics. In 1948 it was demonstrated that a number of antihistamines possessed some pharmacologic properties in common with atropine, procaine amide and quinidine. By 1952 the suppression of ventricular premature systoles by antazoline had been described, and in 1959 a report appeared noting that antazoline was more effective than quinidine in the suppression of spontaneous and surgically induced ventricular fibrillation in the hypothermic dog.[30] In common with quinidine, antazoline is a local anesthetic, possesses some anticholinergic properties and causes mild adrenergic blockade. However, as opposed to quinidine, it increases peripheral vascular resistance but decreases both stroke volume and cardiac output without altering mean blood pressure. Thus, pressor agents are never required.[26] Like quinidine it produces its antiarrhythmic effects primarily by depressing conduction velocity. Apparently it is effective in atrial, nodal and

ventricular tachycardias, as well as arrhythmias associated with digitalis intoxication.[31] Diphenhydramine has also proven to be an effective antifibrillatory agent at low doses both in the laboratory and clinic.[31]

6. Central Nervous System (CNS) Agents. Diphenylhydantoin (Dilantin®) is a potent antiepileptic which produces generalized depression of the CNS. It has been used successfully in abolishing numerous arrhythmias both in the laboratory and in the clinic.[31,34-37] Its duration of action is however, relatively short (2 - 30 mins.). In the heart Dilantin is said to diminish conduction velocity, may depress contractility and produces a net efflux of intracellular sodium and an influx of potassium, which tends to raise the resting membrane potential thereby reducing the likelihood of cardiac arrhythmias. The general impression is that it should not be used as an antiarrhythmic until procaine amide and quinidine have been tried first.

Fekete and Borsy[38] have shown that certain thymoleptics, such as imipramine, desmethylimipramine, amitriptyline and trimepropimine display mild antiarrhythmic activity in rats and dogs.

7. Miscellaneous Agents. Space limitations preclude the inclusion of the numerous substances that have been tested for antiarrhythmic activity. The antimalarial substance chloroquine, depresses myocardial excitability and exerts antifibrillatory activity as does the oral hypoglycemic phenformin (DBI®).[33] Sparteine is a potent, short acting antifibrillatory substance which, in contrast to quinidine, increases myocardial conduction velocity.[33] Much interest has been generated in the antiarrhythmic properties of synthetic oxytocin (Syntocinon®) especially by Canadian workers. Syntocinon has been demonstrated to suppress a variety of laboratory and clinical arrythmias, including those related to catecholamines as well as arrhythmias induced by injecting metrazol into the fourth ventricle.[39-43] It should be noted however, that 0.5% chlorobutanol is used in the vehicle and that it also displays antiarrhythmic activity (unpublished observations). The cholinergic drug edrophonium chloride (Tensilon®) has been reported to display transient antiarrhythmic activity, eliminating paroxysmal auricular tachycardia in about 50% of treated patients.[44] Russian workers have recently described the use of cocarboxylase as being effective against a variety of clinically observed arrthythmias.[45] Iproveratril (Isoptin®) is a new beta adrenergic blocker that has been claimed to possess antiarrhythmic activity greater than either pronethalol or MJ 1999.[46,47] However, it is also a toxic substance with a rather narrow margin of safety.

Chelating agents, such as EDTA, have been employed with various degrees of success in the past. A new substance in this group is 10-phenanthroline which was reported to be as effective as quinidine in reversing auricular fibrillation of isolated rabbit hearts. Apparently it accomplishes this by lengthening the refractory period and the depolarization period.[48,49]

Newer chemical moieties which have been reported to possess antiarrhythmic activity, but which cannot be critically evaluated at this time, are:

N,N-diisopropyl-N' -isoamyl-N' -diethylaminourea (P-286)[50,51], a series of N-(ω-aminoalkyl)phthalimides[52-55], the procaine amide analogs of p-amino-N-[2-(substituted amino) ethyl] benzamides,[56] derivatives of morphanthridine[57], 2-diethylamino-1-phenylethyl, 2-ethoxy HCl and its 4-ethoxy analog[58], and the local anesthetic N,N-bis (phenylcarbamoyl-methyl) dimethylammonium Cl (QX-572)[59]. More work will have to be done on these substances before a fair pharmacologic appraisal can be made.

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Chapter 9. Pulmonary and Antiallergy Agents.

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Medicinal chemical progress in the fields of pulmonary and antiallergy agents* has been slow in recent years. At least three factors appear to have been responsible, in varying degrees, for this hiatus: (1) there have been relatively few advances in insight into the nature of underlying mechanisms, so that screening methods have tended to remain unspecific or inappropriate; (2) the pursuit of existing structural leads has failed to provide agents superior to those already in use; and (3) in some instances there has not been an obvious need to improve upon existing drugs, especially in the face of more urgent problems in other areas, which has tended to discourage specific research calculated to produce new leads and new methodology. Retrospectively, this pattern was substantially unchanged in 1965, and adequate summaries of current status are provided in appropriate sections of newly revised editions of standard reference texts.[1,2]

The field of pulmonary agents seems certain to become considerably more active in the future, however, in the light of clinical developments that have received widespread attention in 1965. The National Heart Institute, in a report to the Senate Appropriations Subcommittee,[3] stated that "emphysema has mushroomed into a major health problem, seemingly overnight...disability benefits for emphysema are (now) exceeded only by disability benefits for atherosclerosis and coronary heart disease." Many clinical discussions of chronic obstructive lung disease presented similar conclusions.[4] Inspection of the agents presently available to the physician shows the list[5] to be dominated by sympathomimetic and xanthine bronchodilators, expectorants and mucolytic agents - and none of these is particularly new. (Certain respiratory stimulants[3,6] and antiinflammatory agents[3] are also reported, but their use is not universally favored.[4,7]) The entire field was thoroughly reviewed by Aviado in two volumes citing several thousand references.[8] It is clear that there is room for improvement in all classes, and it may be expected that other approaches to complement or displace those in current use will emerge as research interest increases.

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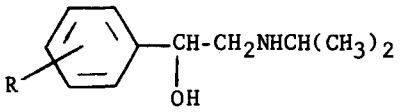
*For the purpose of this review, the category "pulmonary and antiallergy agents" is considered to include bronchodilators, antitussives, expectorants, mucolytics, nasal decongestants and antipruritics (antihistamines). Antibiotics, which occupy an important therapeutic position in the respiratory area, and antiinflammatory agents are excluded because they usually would not be developed specifically for these indications (although antiinflammatory steroids designed for topical use might constitute an important exception). Desensitization procedures are considered to be outside the scope of medicinal chemistry.

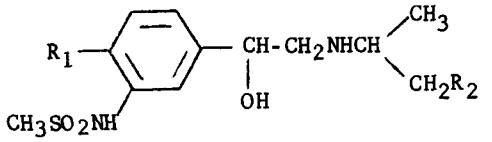
There can be little doubt that bronchodilation by sympathomimetic agents is mediated through β-receptors, so that it is not surprising that side effects such as tachycardia, resulting from excessive stimulation of β-receptors at other sites, should be a common occurrence.[1a,2a] Poor or erratic oral absorption often presents an additional problem. Isoproterenol (I), the prototype β-sympathomimetic, dominates the group.[3,4] Most of the newer agents are closely related to isoproterenol,[5] and all are most often administered locally in some form of aerosol to minimize side effects. Two additional analogs, clorprenaline (II)[9] and metaproterenol (orciprenaline, III),[10] received considerable clinical attention, especially in Europe. The former is suitable for oral administration, but



	R
I	3,4-(OH)2
II	2-Cl
III	3,5-(OH)2

it appears to elicit sufficient CNS stimulation to require concomitant administration of a CNS depressant. The latter, which is reported to have a minimal effect on heart rate, has been most often administered by aerosol.

A very recent report[11] described a series of catecholamine analogs in which the phenolic hydroxyls are replaced by alkyl- or arylsulfonamido substituents of comparable acidity. When this substitution is meta to the ethanolamine side chain, potent α- or β-receptor stimulants result; the most active β-agonists of the series (IV, V and VI) approach the potency of epinephrine or isoproterenol in smooth muscle preparations. Bronchodilator studies with these analogs have not been reported, and little can be discerned from present evidence regarding specificity of action. Interestingly, comparable substitution in the para position



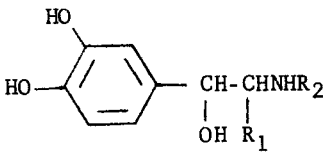
	R_1	R_2
IV	H	H
V	OH	H
VI	OH	$CH_2OC_6H_5$

affords predominantly β-blocking agents.

The problem of specificity of action among β-sympathomimetics remains a substantial challenge, which many would argue to be essentially unresolvable because of the wide anatomical distribution of β-receptors. However, the work of Lands and Brown,[12] showing that α-ethyl substitution in certain β-agonists markedly favors β-receptors in the bronchioles over those in the heart (Table I), suggests that differentiation among β-receptors is possible and that careful study may reveal considerable opportunity to achieve specificity for a particular site.

Table I

Effect of α-Ethyl Substitution on the Specificity of Action of Sympathomimetic Amines[12]



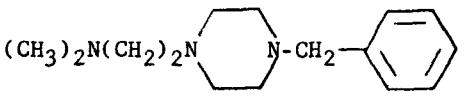
R_1	R_2	Relative Potencies (Isoproterenol = 1) +I[a]	+C[b]	Bronchodilation
H	isopropyl	1	1	1
C_2H_5	isopropyl	0.06	0.001	0.33
H	cyclopentyl	.6	.6	.7
C_2H_5	cyclopentyl	.016	.0005	.45

(a) positive inotropic effect
(b) positive chronotropic effect

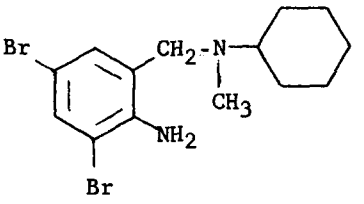
Over the years, a considerable number of xanthine analogs and derivatives have been studied. However, none has yet succeeded in displacing theophylline, which remains the predominant non-sympathomimetic bronchodilator,[3-5] in spite of substantial oral absorption and side effect problems.[1b,2l] Because of the apparent structural and pharmacological limitations inherent in the sympathomimetic approach, the future seems certain to bring a substantial increase in efforts to develop agents more closely related to the xanthines. A novel working hypothesis, which derives from the elegant work of Sutherland and his group,[13] offers a possible new approach to the development of improved agents in this class. Although there is some evidence that is not entirely consistent,[1b] the hypothesis suggests that the bronchodilator action of theophylline is the result of increased levels of cyclic 3',5-AMP brought about by phosphodiesterase inhibition. This would directly relate theophylline to β-receptors, where cyclic 3',5'-AMP synthesis is thought to be increased through stimulation of adenyl cyclase by appropriate agonists. There is yet little direct evidence, however, to suggest that this is the only, or even the major, mechanistic component of theophylline bronchodilation, so it remains to be determined how fruitful the approach will be.

Mucolytic agents and expectorants[1c,2c] are used to decrease the viscosity of, and aid in clearing bronchial secretions. A number of agents are available,[5] including many older preparations such as iodides and guaiacolates. The more recent additions, N-acetyleysteine, ribonuclease and various proteolytic enzymes, are usually administered locally in a nebulized form. The clinical value of these agents does not appear to be unanimously accepted,[4] and objective demonstration of efficacy is often difficult to obtain.[14] This suggests a decided need for more effective products, since there seems to be little doubt that this is a valuable therapeutic approach.[3,4]

Better agents with local action, designed from chemical knowledge of specific components in bronchial secretions,[15] represent an obvious avenue of attack. The reports that pimetine (VIII)[16] and Bisolvon (IX)[17] reduce sputum viscosity after systemic administration are probably of greater potential interest, since agents not dependent on delivery through airways that may be severely obstructed would be expected to have inherent advantages.



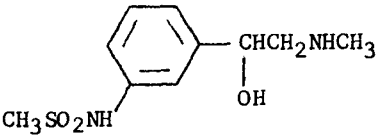
VIII



IX

Antitussive drugs[1c,2c] were reviewed by Bucher,[18] who clearly pointed out the need for more sophisticated screening tools based on a better understanding of the cough reflex. Antitussive activity in several structural types was reported,[19-25] but these do not appear to represent substantial departures from those reviewed earlier by Chappel and von Seeman.[26]

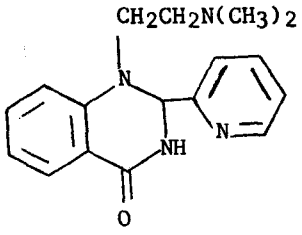
Drug treatment of allergic disorders, if antiinflammatory agents are excluded, is limited almost exclusively to antihistamines[1d,2d] and certain sympathomimetic agents.[1a,2a] The latter class includes both α-mimetics, used as nasal decongestants, and β-mimetics (discussed above), which are generally for acute use as in anaphylactic reactions. A new α-mimetic, amidephrine (X), from the series of sulfonamido analogs already mentioned, was reported to be an effective and long-acting nasal decongestant.[11,2]



X

The complexity of allergic phenomena, and the need for true understanding of the interplay of the various contributing factors - histamine, serotonin, kinins and others - is apparent in the mechanistic studies[28] that continue to investigate various aspects of hypersensitivity and especially in the review of methods for the induction of experimental hypersensitivity by Spencer and West.[29] A symposium chaired by Ungar explored the variety of physiological functions proposed for histamine,[30] and Garattini and Valzelli presented an extensive review of serotonin.[31] Cursory reviews of present antihistamine usage[32] served to emphasize the relative quiescence of the field in terms of drug development.

Various 6-7-6 tricyclic ring systems - structurally the most interesting class of antihistamines, even though their impact has been far greater in other fields - continued to receive attention. Structure-activity studies in dibenzocycloheptene analogs related to cyproheptadine were reported.[33] Rubin described the pharmacology of a dibenzoxazepine with 1-10 times the potency of pyrilamine,[34] and Protiva reviewed recent developments in several other, related systems.[35] In other areas, a series of tetrahydroquinazolines was described,[36] one of which (XI) was reported to be comparable to chlorpheniramine in the clinic.[37]



XI

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Chapter 10. Agents Affecting Gastrointestinal Functions

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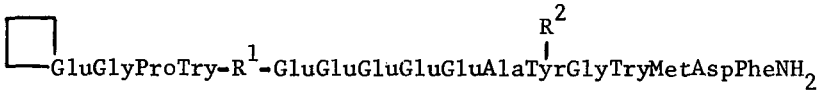
EXPERIMENTAL INVESTIGATIONS

Stimulation of Gastric Secretion

Gastrin: A major advance in the biochemistry of gastric secretion has been made with the determination of structure and synthesis of gastrin I and II from various species. Porcine gastrin I has been shown to be the heptadecapeptide I. Porcine gastrin II (II) has the same peptide sequence but with



I, R^1 = Met, R^2 = H III, R^1 = Leu, R^2 = H

II, R^1 = Met, R^2 = SO_3H IV, R^1 = Leu, R^2 = SO_3H

the tyrosine hydroxyl in the form of its sulfate ester.[1] Both were equipotent and showed the same biological effects. The pure human gastrins have been isolated,[2] and degradative analyses[3] and total synthesis[4] showed that human gastrin I (III) and human gastrin II (IV) were heptadecapeptides identical to the porcine gastrins except for replacement of one of the methionine components by leucine. The porcine gastrins stimulated secretion in other species including dogs and man.

Elucidation of the amino acid sequence of the gastrins permitted an examination of the effect of structure modification on the biological activity and presented an opportunity for the synthesis of inhibitory analogs of the natural hormone. A series of peptides related to gastrin I has been tested.[5] The C-terminal tetrapeptide (V) was the minimum fragment of the gastrin molecule which

V, TryMetAspPheNH$_2$

showed all the physiological activities of the natural hormone. Based on stimulation of gastric acid secretion in dogs, it was about 20% as potent as gastrin. The C-terminal amide was essential for activity, whereas acylation of the N-terminal amino group had little effect on the activity.

The effect on activity of a number of structural modifications of the tetrapeptide (V) has been determined.[6] The variations in the main group of analogs studied consisted of single or multiple substitution of one or more of the amino acid residues, including replacement of the normal L amino acids by D amino acids. Activity of products with respect to gastric secretion in dogs varied from equipotent with gastrin for certain acylated derivatives (VI, VII)

VI, $H_2NCOTryMetAspPheNH_2$

VII, $(CH_3)_3COCO$-β-AlaTryMetAspPheNH$_2$

to inactive for those peptides not having a terminal amide group. In addition, the effect of some of the analogs on other physiological functions such as gastric motility was not uniformly consistent with the direction of the effect on gastric secretion, thus indicating a separation of the various gastrin activities. The presence of D amino acids gave peptides of decreased activity, and none of these products, including the inactive all D tetrapeptide, inhibited the action of gastrin. It was concluded that the most important structural feature of the tetrapeptide for activity was the aspartic carboxyl-terminal amide combination.

In humans, gastrin was 80-fold more potent than histamine in stimulating secretion.[7] At present, the major clinical use of gastrin or active fragments thereof would appear to be for the replacement of histamine and other secretagogues for diagnostic analysis of gastric secretions.[7-9]

Gastrin appeared to exert a stimulatory and inhibitory effect on acid secretion, at low and high doses, respectively, and a stimulatory effect on pepsin secretion at high doses regardless of whether the initial stimulation was histamine or gastrin.[5,10] However, it has been suggested that stimulation was the normal effect and inhibition was the result of overdosage.[11] The histamine content of the stomach mucosa was decreased by gastrin, indicating that it may act as a secretory agent by releasing histamine.[12] Acid secretion stimulated by exogenous gastrin was inhibited by heparin[13] and by insulin hypoglycemia.[14]

Gastrin II has been shown to increase oxygen consumption and cause vasodilation in the stomach mucosa.[15] It also caused a pronounced increase in gastrointestinal motility which was inhibited by atropine.[16] Gastrin I and II were approximately 20-fold more active than histamine for the stimulation of intrinsic factor secretion by the human stomach.[9]

2-Deoxyglucose: Study of acid secretion stimulated by 2-deoxyglucose indicated that it functions by forming nonmetabolizable 2-deoxyglucose-6-phosphate, resulting in cytoglucopenia at the vagal secretory center. Thus, in effect, this mechanism of stimulation of gastric secretion was apparently equivalent to the hypoglycemic mechanism of stimulation resulting from insulin administration.[17] It has recently been shown that, after insulin stimulation, the increase in flow of gastric contents occurred simultaneously with or followed the lowest point reached by the blood sugar.[18]

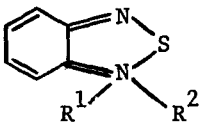
Since the central regulatory mechanism for gastric secretion acts through the vagus nerve, stimulation of the secretory center by insulin is a method which has been used to study the completeness of vagotomy in the surgical treatment of peptic ulcer. Tests in humans showed that 2-deoxyglucose produced the same hypersecretion as insulin without the attendant side effects. Therefore, it has been suggested that insulin be replaced by 2-deoxyglucose for evaluation of patients who have undergone vagotomy.[19]

Histamine: Numerous studies devoted to the evaluation of the role of histamine in stimulating gastric secretion have been reported. All results indicated that histamine is the chemical mediator of gastric secretion common to all stimulants. Inhibition of specific histidine decarboxylase by the hydrazino analog of histidine and NSD-1055 (4-bromo-3-hydroxybenzyloxyamine), agents which lower tissue histamine levels,[20] in pylorus-esophagus-ligated rats prevented stimulation of acid secretion by reserpine, bethanechol chloride (2-carbamoylpropyltrimethylammonium chloride), insulin and gastrin, but not exogenous histamine. These results showed that acid secretion was dependent on the presence of histamine in the mucosa.[21] It has also been shown that vagal stimulation led to mobilization of gastric histamine.[22] Gastric secretagogues, such as gastrin, insulin, betazole [3-(β-aminoethyl)pyrazole] and bethanechol chloride, lowered gastric tissue histamine levels, but it was not known if their stimulatory effect on secretion was a consequence of gastric histamine release. However, the enzyme histaminase prevented gastric acid secretion in dogs under the stimulating effect of a gastrin extract.[23]

Portacaval shunt, which in man results in an increased incidence of duodenal ulcer, in rats caused an increase in acid secretion. Also, histidine decarboxylase activity in the fundus, the acid-secreting portion of the stomach, has been shown to undergo a 4-fold increase after this operation. The elevated acid secretion was reduced essentially to normal by treatment with NSD-1055, the previously mentioned inhibitor of histidine decarboxylase.[24]

Inhibition of Gastric Secretion

Benzothiazoles: Antisecretory activity in a series of 3-amino-2,1-benzisothiazoles (VIII), determined by measuring gastric volume reduction after

VIII, 

subcutaneous administration to pylorus-ligated rats, varied from an ED_{50} of 1.5 mg./kg. to greater than 50 mg./kg. R^1 and R^2 consist of hydrogen, lower alkyl and phenyl, and some members of the series were also substituted in the benzenoid ring. Representative examples from the series did not show anticholinergic activity.[25]

Anticholinergics: The effects of anticholinergic agents on gastric acid secretion were compared with pharmacological effects on gastric emptying and pupil diameter, and it was concluded that the relationship of the secondary actions to the desired action of gastric acid inhibition was more important in evaluation than the absolute potency.[26]

Experimental Ulcer Production

The major objectives of the examination in laboratory animals of agents which produced gastrointestinal irritation or ulceration have been the discovery of preventative or counteracting agents and elucidation of the mechanism

of ulceration. The ulcerative action of serotonin was significantly diminished by administration of the antiserotonin, methysergide (Deseril; 1-methyl-D-lysergic acid butanolamide tartrate).[27,28] Antienzymes of pancreatic origin (Kunitz inhibitor)[29] or parotid origin (Frey inhibitor)[30] markedly reduced the number of lesions resulting from reserpine or phenylbutazone.

Aspirin damage to the intestinal mucosa occurred regardless of whether contact with the drug was from the lumen or the circulation.[31] It has been shown that aspirin greatly increased the ionic permeability of the mucosa and broke down its normal barrier to diffusion of sodium, potassium and hydrogen ions, so that absorption of hydrogen ions through the damaged barrier from the stomach led to mucosal bleeding. During active secretion of acid these effects may be enhanced.[32,33] It has also been proposed that aspirin decreased the rate of formation of the mucous lining and also lowered its resistance to proteolysis.[34]

Acetazolamide damage to the mucosa appeared to depend upon the presence of acid and, in addition, it accelerated the loss of hydrogen ions from the luminal fluid through the surface mucosa.[35] Enhancement of gastric secretion was suggested to be a part of the mode of action of caffeine in ulcerogenesis.[36]

The polysaccharide carrageenin has been found to have a protective effect from ulceration induced by histamine, cortisone or the Shay technique.[37,38]

Absorption and Transport

Most experimental studies on absorption and transport were carried out to determine mechanism of these actions rather than to search for agents affecting *in vivo* action. Much of the work was done in *in vitro* gut sac preparations. Stimulation of glucose and sodium transport by reserpine resulted, at least in part, as a result of release of catecholamines from storage sites in the intestine.[39] Similarly, L-epinephrine and L-norepinephrine stimulated glucose and sodium absorption, but stimulation was blocked by prior treatment with dichloroisoproterenol or ergotamine, although the latter two agents alone had no effect. Cyclic AMP also had no effect.[40] It was concluded that calcium binding substances reduced gastrointestinal absorption of drugs mainly by markedly decreasing the rate of gastric emptying.[41]

Transport of amino acids across the intestinal wall was inhibited by 4-deoxypyridoxine, a vitamin B_6 antagonist, whereas glucose transport was unaffected. It was concluded that vitamin B_6 may be necessary for a metabolic process involving the amino acid carrier systems.[42]

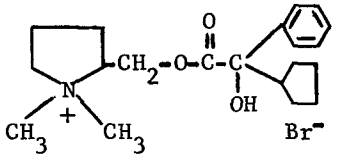
CLINICAL INVESTIGATIONS

Peptic Ulcer Therapy

Anticholinergics: Although anticholinergic drugs are prescribed for the inhibition of gastric acidity in peptic ulcer disease, there was no indication that average doses of these agents significantly inhibited gastric acid secre-

tion.[43] Thus, for any significant degree of inhibition of gastric secretion, an anticholinergic agent must be administered at the maximally tolerated level.[44] However, combinations of antacids and anticholinergics were found to be more effective in reducing gastric acidity than either drug given alone. Several new anticholinergics have undergone clinical trial, but none appeared to be any more potent or selective in action than atropine.[45,46] Glycopyrrolate (1-methyl-3-pyrrolidyl α-phenylcyclopentaneglycolate methobromide)(IX) has been

IX,



shown to be a clinically useful anticholinergic,[47,48] but the drug had no special qualities which distinguished it from the more than 50 anticholinergics now available. The α-phenylcyclohexaneglycolate congener of glycopyrrolate, which was equiactive in animals, was found to be only minimally effective in suppressing gastric acidity in man.[49]

Antacids: These agents are widely used to reduce gastric acidity and relieve pain in the peptic ulcer patient. The mechanism of pain relief was unclear, since new telemetry techniques for measuring gastric pH[50] have confirmed the older reports that the acid-neutralizing action lasted only from 30 to 40 minutes, while pain relief lasted for hours. A new antacid, bismuth aluminate, has been reported to be effective in relief of pain, but this agent had no effect on gastric acidity or peptic activity.[51] The effect was postulated to be due to a coating action of the gastric mucosa.[52] As with anticholinergics, the antacids[53] gave symptomatic relief, but did not alter the natural course of the disease.

Antipeptic Agents: Since acid-pepsin digestion is considered to be essential for the production of peptic ulceration, regardless of the underlying cause of the disease, reduction of pepsin concentration of gastric juice remains a practical aim in ulcer therapy. Sun[54] has reviewed this area with particular attention to a new antipeptic agent, Depepsen, a sulfated amylopectin. The place of these agents in peptic ulcer treatment is still controversial.

Gastric Ulcer Therapy

Gastric ulcer patients differ from those with duodenal ulcer, in that the former have normal or low gastric acidity and a much higher incidence of gastric cancer. Carbenoxolone (Biogastrone), the pentacyclic triterpene, glycyrrhetinic acid, has been reported to give symptomatic relief in gastric ulcer patients and to increase the rate of gastric ulcer healing, confirming the original clinical studies.[55-57] Some investigators, however, have failed to find effects better than placebo,[58] and side effects related to salt and water retention were common. The mechanism of the antiulcer action was not clear, but could be related to the drug's antiinflammatory effects.

Functional Gastrointestinal Disorders

Anticholinergics have been used for treatment of hypermotility states with some success, but with the usual side effects. A large series of thioxanthene derivatives was studied for selective anticholinergic action, and 9-[(N-methyl-3-piperidyl)methyl]thioxanthene, marketed as methixene (Trest)(X), has been found to provide symptomatic relief with low incidence of atropine-like side

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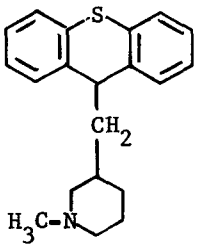

effects.[59,60] It is too early to tell if this drug will replace traditional anticholinergic treatment in hypermotility states.

In the carcinoid syndrome, it has been suggested that serotonin was responsible for the diarrhea seen in this condition. Treatment with a serotonin antagonist,[61] methysergide, was effective in controlling the diarrhea and was further evidence for the role of serotonin in intestinal motility.

The search for agents which alter smooth muscle activity yielded several new compounds. Antispasmodic effects were reported for pyrrole,[62] acetophenone[63] and piperidine derivatives,[64] as well as dehydromethysticum[65] and 2-(2'-phenylacetylhydrazino)-4,6-bisdiethylamino-1,3,5-triazine (Ciba 28,882-Ba).[66] The spasmogenic effect of dihydrocodeine was reversed by nicotinic acid esterification.[67] Nicotinyldihydrocodeine was found to inhibit drug-induced spasm of the bile duct sphincter. Stimulation of smooth muscle activity was reported for tetrahydroaminoacridine compounds.[68]

Metoclopramide [N-(2-diethylaminoethyl)-2-methoxy-4-amino-5-chlorobenzamide dihydrochloride] (XI) has been widely used in Europe as a "digestive

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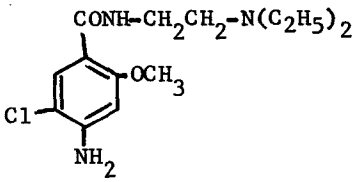

modifier" to relax the pylorus and increase gastric motility and as an aid to the x-ray study[69] of the gastrointestinal tract. Its primary use has been as an antiemetic. At the present time, this drug is not available in the United States.

Ulcerative Colitis Therapy

The choice between medical and surgical treatment of this disease remains a problem to the clinician. Reviews of the literature[70,71] indicated that steroids were the most commonly used drugs; however, as with anticholinergic therapy for peptic ulcer, they alleviated the symptoms but did not cure the disease.

Drug Effects on Gastrointestinal Motility

Stimulation of gastrointestinal motility has been studied, usually with isolated tissues, with a wide variety of drugs. Serotonin has been suggested to be a naturally occurring material which stimulates motility, perhaps as a local hormone. However, its role remained unclear, since it has been shown to stimulate human tissues *in vitro*,[72] but up to 90% depletion in the rat did not inhibit peristalsis.[73] A study on the isolated rat colon indicated that the effect of serotonin was dose-related: small doses had a direct effect on muscle fibers, while higher doses acted by stimulating parasympathetic ganglia in the gut wall.[74]

Bile

Drug-induced jaundice as a side effect of phenothiazine derivatives is well documented. The effect of these drugs and $C_{17\alpha}$-alkyl-substituted steroids was studied by *in vivo* and *in vitro* tests,[75] and it was concluded that these agents inhibited glucuronyl transferase. The steroids prevented excretion of conjugated bilirubin by blocking a stage after conjugation, but the mechanism of phenothiazine jaundice was still unclear. The steatorrhea observed in patients receiving cholestyramine (a basic ion exchange resin which binds bile acids) and a diet containing triglyceride fats of normal chain length was eliminated by replacing the long chain triglycerides with medium chain triglycerides (less than C_{12}). Bile acid absorption by the cholestyramine was comparable for both diets. The medium chain triglycerides may be of value in clinical management of patients with malabsorption due to biliary obstruction.[76]

Salivary Glands

Physalaemin, a polypeptide, was found to be the most active sialogogic agent tested in rats and dogs.[77] Its action appeared to be a direct one on the salivary gland, since its effect was not blocked by sympathomimetic, parasympathomimetic or ganglionic blockers, but its effect was reduced by serotonin.

Drug-Induced Gastrointestinal Lesions

The incidence of pathological changes in the gastrointestinal tract due to therapeutic agents has increased as new drugs and formulations appeared on the market. Gastrointestinal ulceration due to steroids and antiinflammatory drugs is well known and continues to be a major side effect. Reduction of ulcerogenic activity was reported with ketophenylbutazone as compared to phenylbutazone itself,[78] while the use of indomethacin capsules reduced the incidence of peptic ulcers seen with indomethacin tablets.[79] The mechanism of steroid-

induced gastrointestinal lesions is still not resolved. Hydrocortisone did not affect gastric secretion in the rat, leading to the conclusion that some mechanism other than increased gastric acidity was the cause of corticosteroid ulceration.[80,81] There did not seem to be a specific steroid ulcer[82] as distinguished from ulcers due to other causes. It has been suggested[83] that steroids aggravated existing inflammation by loosening intercellular cement and permitting penetration of the mucosa by the gastric juice. Steroid therapy in cancer patients appeared to induce more severe gastric complications than in patients with nonmalignant disease.[84]

A new iatrogenic lesion, small bowel ulceration following administration of enteric-coated tablets of potassium chloride and a thiazide diuretic, was described in late 1964[85] and rapidly confirmed by numerous case reports.[86-89] Experimental studies indicated that the lesion was due to the release of a high local concentration of potassium from the enteric-coated tablet over a short segment of the intestine.[90] In one clinical report,[91] a partially digested tablet was found in a lesion crater in the intestine. The thiazide diuretic did not appear to be connected with the lesion production.[92,93]

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Section III - Chemotherapeutic Agents

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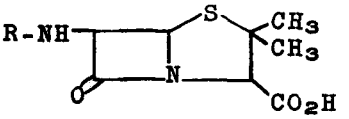
Chapter 11. Antibiotics and Related Compounds

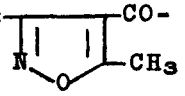
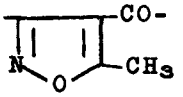
Edwin H. Flynn, Eli Lilly and Co., Indianapolis, Ind.

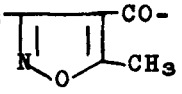
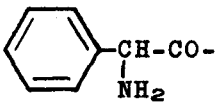
The year 1965 was marked by appearance of a number of valuable reports bearing on the use of antibiotics in the chemotherapy of infectious diseases. New advances have been made in understanding the biosynthesis of some agents and experiments have been reported which were designed to make the mode of action understandable. Although not all-inclusive, papers referred to in the following pages should give the reader a starting point for further reading and may indicate the directions being taken by research in this area.

Penicillins

New derivatives of 6-aminopenicillanic acid (I) continue to



I, R = H; II, R =  ; III, R = <u>o</u>-ClC$_6$H$_5$ 

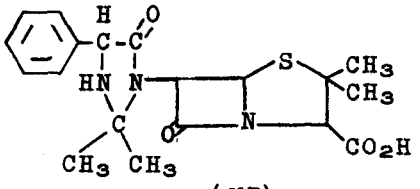
IV, R = 2,4diClC$_6$H$_5$  ; V, R = <u>D</u>- 

find a place both in experimental work on infectious disease and in medical practice. Two derivatives closely related to oxacillin (II) are cloxacillin (III) and dicloxacillin (IV). These compounds, though described earlier, have been studied further. It has been said[1] that cloxacillin is primarily useful in infections caused by penicillinase producing staphylococci. Blood levels after oral and intramuscular administration were slightly more delayed when compared with oxacillin but lasted longer. Peak levels were thought to be similar, although other workers[2] have reported better absorption than that obtained with oxacillin. A clinical study[2] of cloxacillin showed good results in staphylococcal, pneumococcal and streptococcal infections and consistently higher antibacterial blood levels

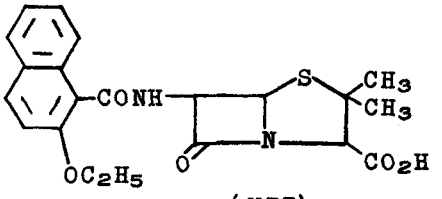
after oral administration. It was concluded that cloxacillin could substitute for methicillin when oral therapy is feasible. Dicloxacillin appears to be absorbed extremely well, giving peak blood levels twice those of cloxacillin and four-fold greater than oxacillin.[3] It was felt that serum binding was comparable to the other isoxazolyl penicillins. These blood levels have been confirmed.[4]

Ampicillin (V), known to be absorbed well orally and to have a broader spectrum of activity than other penicillins, has been studied as the sodium salt for parenteral administration.[5] Results indicated this form to be satisfactory for parenteral use, thus enhancing the clinical value of ampicillin.

Hetacillin (VI), the reaction product of ampicillin and acetone, has been reported.[6] It is said to give more prolonged blood levels after oral dosage than does ampicillin, and apparently is active *in vivo* by virtue of hydrolysis to ampicillin.[7]



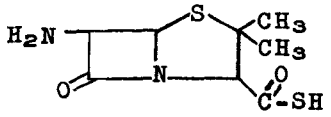
(VI)



(VII)

Nafcillin (VII) has been reported to be an effective and well tolerated antistaphylococcal agent comparable to other penicillinase resistant penicillins.[8]

N-alkyl derivatives of phenoxymethyl penicillin (penicillin V) have been described and turn out to be essentially inactive as antibacterial substances when compared with the unsubstituted compound.[9] Active derivatives of 6-aminothiopenicillanic acid (VIII) have been reported.[10] An interesting series is that represented by IX, this member showing good gram negative as well as gram positive activity.[11]



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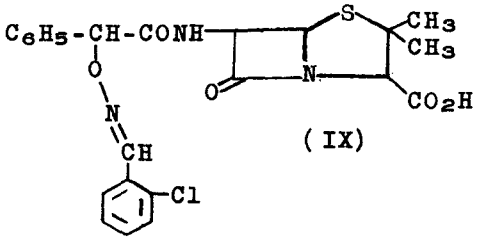


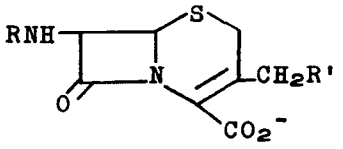
(IX)

A proposal concerning the mechanism of action of penicillins on the bacterial cell has been advanced.[12] An interesting paper concerning the initial structural lesions produced by penicillin acting on the bacterial cell has appeared.[13] It verifies a highly specific point of attack by this antibiotic.

A worthwhile review of penicillins has appeared[14] and a book has been published[15] which is concerned primarily with biochemical and biological factors related to penicillin.

Cephalosporins

The cephalosporin group of antibiotics, whose genesis may be traced to the discovery of cephalosporin C (X), has demonstrated their value in the treatment of infectious disease. Cephalothin (XI), the first of these substances to find widespread use, was followed by cephaloridine (XII) which is now employed in many countries for therapy of bacterial infections. It seems apparent that other valuable compounds of this class may be expected.



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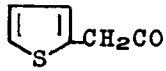
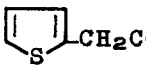
XI, R = 

, R' = CH_3CO_2-;

XII, R = 

, R' = 

XIII, R = D-$C_6H_5CHNH_2CO$-, R' = CH_3CO_2-

Cephaloridine has been evaluated in a number of clinical and laboratory studies;[16-18] other reports not cited here may be found in the Abstracts of the Fifth Interscience Congress on Antimicrobial Agents and Chemotherapy. It can be said in general that the agent was effective in a variety of both gram negative and gram positive bacterial infections. Cephaloridine was ineffective against Pseudomonas, the tubercle bacillus, and fungi. In a comparison with cephalothin significant differences in therapeutic response were not detected.[17] Generally satisfactory results were seen in a pediatric study[18] of cephaloridine. A series of 92 cases with a variety of infections was studied with impressive results.[19] Two cases of renal failure were encountered while under treatment with 8-12 grams of cephaloridine daily; the complex hospital courses did not allow direct implication of the drug. In another series of 70 cases at what would seem to be low daily doses, good results were reported.[20] The same report mentions toxic changes in rabbit kidney at a single dose of 90 mg./kg. but not at 45 mg./kg. daily for eight weeks. All of the foregoing studies employed parenteral administration.

The bactericidal activity of cephaloridine has been commented upon favorably[21] and its stability to staphylococcal penicillinase has been studied.[22,23] Allergy to cephaloridine has received notice[24] as has a case of anaphylaxis attributed to cephalothin.[25]

An interesting investigation of synergism between penicillins and cephalosporins against Ps. pyocyanea has been published showing marked enhancement of activity in certain cases.[26]

Cephaloglycin (XIII) has been evaluated[27] in the laboratory both in vitro and in vivo and shows a good level of activity versus gram negative organisms. The article points out some difficulties inherent in evaluating a substance which is relatively unstable in solution as is the case with cephaloglycin.

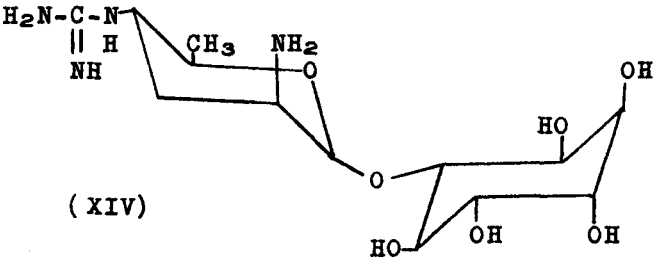
Chemical papers have appeared, one describing physical chemical data on a series of cephalosporins[28] and another mentioning preparation of a number of derivatives.[29]

Lincomycin

Lincomycin, an antibiotic effective in treatment of infections caused by gram positive organisms, is finding widespread use in the treatment of human disease. Earlier reports (1963 and later) demonstrating clinical efficacy have been extended.[30-31] That structural modification of lincomycin is possible without loss of antibacterial activity has been shown in several papers.[32,33,34] Some studies on biosynthesis have been reported.[35]

Potpourri

Several reports concerned with a new entity called kasugamycin have appeared. This substance has been shown to have the structure, XIV.[36] Originally discovered because of its



(XIV)

activity in P. oryzae infections of the rice plant, it has antibacterial activity of a fairly wide range, though of a low order.[37] Orally absorbed, and apparently relatively non-toxic, kasugamycin may find application in treatment of human infections. Its utility in Pseudomonas infections of the genitourinary tract has been suggested in one paper.[38]

Derivatives of rifamycin have been reported[39,40] and at least one compound has been shown to be effective in human disease.[41] The diethylamide of rifamycin B has shown effectiveness in acute cholecystitis and in staphylococcal infections. Less favorable results were obtained in pneumonia. A review has appeared on rifamycin SV.[42]

Additional information on the gentamicin complex, first reported in 1963, has been made available during 1965. This multiple factor agent(s) has been shown to have value in the treatment of Pseudomonas sepsis in burns[43] and the (rabbit) eye[44] and has been used in pediatrics to treat gram negative infections.[45] Although somewhat toxic, gentamicin appears to have clinical value. Some chemical studies have been reported,[46] showing the presence in the molecule of D-glucosamine, 2-deoxystreptamine and an amino sugar named gentosamine A.

A conference to discuss new antituberculous agents was held by the New York Academy of Sciences September 13-15, 1965, in New York City. The use of ethambutol and capreomycin in therapy was discussed.

Actinospectacin, an agent with broad spectrum activity,[47] showed promise in acute infections of the genitourinary tract.[48] The relatively low percentage of "good" responses was probably due to inadequate dosage.

Isolation and biological assessment of coumermycin has been reported.[49] The structure has been established[50] and its synthesis from a novobiocin intermediate has been mentioned.[51] Chemically related to novobiocin, coumermycin A_1 shows cross resistance with novobiocin; it appears to be more active in general. It is identical to sugordomycin. Coumermycin A_2 is closely related to A_1 chemically.[52]

Chemistry of the polymyxins has been discussed.[53] Synthesis of polymyxin E (colistin A) was the subject of a recent study.[54] Structure studies were described showing the identities of polymyxin E_1 and E_2 with colistin A and B, respectively.[55]

Fusidic acid, the steroidal antibiotic now in clinical use, has been shown to be identical with ramycin.[56] A comprehensive study of the effect of structural variation on antibacterial activity has been reported.[57] The structural relationship with helvolic acid and cephalosporin P_1 is mentioned.

Erythromycin has been shown to be effective clinically[58] in pneumonia due to Mycoplasma pneumoniae, the Eaton agent pneumonia. Biosynthesis and metabolism of erythromycins were studied using 1-^{14}C propionate as precursor.[59] The aglycone was formed and gave erythromycins A, B, and C. A biosynthetic scheme was proposed.

The structures of spiramycin and magnamycin were published.[60] The structure of magnamycin represents a revision of an earlier proposal.

The structure of viomycin has been determined.[61]

Moenomycin, a macromolecule having high intrinsic antibacterial activity *in vitro* and *in vivo* when administered parenterally, has been reported.[62,63,64] The recommendation was made that this interesting substance be reserved for use as an animal growth stimulant.

Tetracycline derivatives have been prepared by alkoxyalkylation of the amide function.[65] The mode(s) of action of tetracyclines have been studied using A. aerogenes.[66] They are said to interfere with hydrogen transfer reactions. A disturbing report of transduction of S. aureus to tetracycline resistance in vivo has appeared.[67] Although a similar circumstance seems remote in humans, the possibility was noted. A further elaboration of biosynthetic pathways for tetracyclines has been provided.[68] The isolation and characterization of 4-hydroxy-6-methylpretetramid, a tetracycline precursor accumulated by a blocked mutant, has been recorded.[69] A new total synthesis of 6-deoxy-6-demethyltetracycline was achieved by an elegant procedure.[70]

The chemistry of the depsipeptide type antibiotics (structures made up of hydroxy- and amino acid moieties linked through ester and amide bonds) and their mechanism of action has been reviewed.[71] A detailed account of the chemistry also has been published.[72] It is thought that these substances act by altering ion transport through cell membranes via changes in the lipoprotein component of the cell membrane.

Some structural aspects of the vancomycin molecule have been defined.[73] Clinically, vancomycin has been shown to be effective in staphylococcal enterocolitis.[74,75]

Lysostaphin, a lytic enzyme preparation specific for the genus Staphylococcus, has been reported to be a mixture of two enzymes, a hexosaminidase and a peptidase.[76] Lytic action is due to the peptidase. Its therapeutic activity in animals has been discussed.[77]

A paper has been published which recognizes the difficulty of determining relative clinical efficacy of antibiotics and proposes possible alternatives.[78] The importance of obtaining an optimum balance of properties in addition to usable antibacterial activity has been neglected by many experts.

A comprehensive study and discussion of serum binding of antibiotics has been published.[79] Still to be correlated experimentally is the suggested deleterious effect on clinical effectiveness with the nature and degree of serum protein binding.

Possible involvement of protoplast forms of bacteria in an infection have been considered. In an experimental approach, evidence was obtained to suggest that a combination of penicillin and kanamycin eliminated both classic and protoplast forms in experimental pyelonephritis.[80] Major advances in infectious disease therapy are destined to originate from studies of the relationship of aberrant bacterial forms to disease.

The biosynthesis of macrolide antibiotics was considered.[81] Aspects of the biogenesis of several other antibiotic groups have been covered in a collection of papers.[82]

A useful review of the chemistry of new antibiotics has appeared.[83]

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Chapter 12. Synthetic Antibacterial Agents

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Developments during 1965 involved primarily laboratory and clinical study of analogs of known antibacterial structures and of their metabolism and mechanism of action. The importance of antibacterials is indicated by the large research effort being expended. The difficulty of finding new antibacterial structures is demonstrated by the fact that only a very few have been reported in this decade in spite of this effort. Most of the 119 years since the beginning (Semmelweiss 1847 and Lister 1865) of modern(1) antibacterial chemotherapy have been spent in stagnation or in significant but slow improvement on the small numbers of different active types so far discovered. Into the attack on the antibacterial frontiers are being brought biochemistry at the enzyme level, molecular vibrations, electronic energy-level calculations and pharmacokinetics via computers but the push-button age of "magic bullets" is still far in the future.

In Vivo Actives.—The new compounds first shown by published data in 1965 to be active against infections in animals are discussed here. A laboratory study(2) on 2-sulfanilamido-4,6-diethyl-s-triazine showed it to be the most active of the sulfatriazines in vivo and the first clinical report was promising(3). It is highly soluble, well absorbed, less conjugated than other clinical sulfas, and excreted largely unchanged(3). Its behavior is between short-acting and long-acting and its renal clearance is uniquely acceleratable by adjustment of urinary pH(3). Chemotherapeutic application of 4-sulfa-1,2,3-thiadiazoles is limited by their explosive intermediates (4). 5-Dimethylsulfamyl-anthranilic hydrazide is only formally a sulfanilamide derivative since it is not antagonized by PAB. The in vivo antibacterial activity(5) is rather specific structurally and is limited to Staphylococcus (ED_{50} 25-50mg/kg) and Pneumococcus (ED_{50} 250mg/kg) but, curiously, shows up in vitro only against Pneumococcus. Low activity against the experimental TB infection in mice was reported for 2-carbethoxyaminoethyl n-butylsulfide(6) and for beta-alanylhydroxamic acid(7). The latter shows some similarities in mechanism of action to cycloserine. Additional data(8) on 1-(aralkoxy and higher alkyloxy)-4,6-diamino-1,2-dihydro-2,2-dimethyl-s-triazines show that some are active in vivo against Strep. hemolyticus but only at just below toxic doses. The broad-spectrum in vitro activity and the toxicity appear to be antifolic in nature. New activity (leprosy, below) was reported for ethambutol and for thalidomide as well as for some azo dyes.

Antituberculous Agents.—Although major advances in TB therapy have been made, treatment is still far from satisfactory and the incidence of new urban cases is increasing, in some instances drastically(9). Ethambutol (dextro-2,2'-ethylenediimino-di-1-butanol), one of two new active types reported in this decade, has successfully undergone clinical trials (10, 11, 12, 13) in several countries. Ethambutol acts clinically (12, 13) and experimentally(14, 15) against strains of mycobacteria resistant to other agents, and combinations of sub-effective concentrations are inhibitory (16) in vitro. Resistant strains, which develop with difficulty(17, 18), have different biochemical characteristics than isoniazid-resistant strains (19). Human and bovine mycobacterial strains, which were experimentally

made resistant to ethambutol, have been found(16) to be attenuated for mice. Using ^{35}S and ^{32}P to estimate protein and nucleic acid synthesis, it has been concluded(19, 20) that ethambutol acts by interfering with a function of cellular polyamines and divalent cations in the synthesis or stabilization of RNA. Preliminary reports of its activity against human(21) and murine(22) leprosy have appeared.

Quickly following a report drawing attention to the carcinogenic activity of isoniazid was a word of caution(23), much needed in the present, somewhat hysterical atmosphere surrounding drugs. The production of tumors only in mice may well have prevented introduction of isoniazid in recent years but should not diminish its use until a harmful effect is established and weighed against its proven merits.

Extensive investigation of N,N'-diaryl and N-aryl-N'-heteroaryl thioureas appears to be ending in naught. N,N'-Bis(4-isoamyloxyphenyl)thiourea, the member most studied clinically, is judged to be only slightly active in man, by the usual criteria(24, 25, 26), on the basis of all the published controlled trials(27) in several countries. In addition, there is a high frequency(28) of natural resistance to this agent and cross-resistance with 4-acetamidobenzalthiosemicarbazone(29).

2-Ethyl isonicotinic thioamide (ethionamide) is undergoing clinical study at decreasing dosage. Although effective(30, 31) in producing clinical improvement and preventing emergence of resistance, high incidence of liver (31, 32, 33) and gastrointestinal(30, 34, 35) toxicity seems likely to restrict its use to retreatment of resistant cases. Mental disturbance and neural effects were cited(31, 36) as reasons for not using it in ambulatory patients. It is converted in man to several metabolites(37), among them the thioamide sulfoxide. It is not cross-resistant with isoniazid but shows some cross-resistance with thiocarbamylhydrazides(thiacetazone) and diarylthioureas(38, 39, 40, 41). Reports on the N-morpholino-methylamide of pyrazinoic acid, proposed as a form of pyrazinamide with decreased toxicity and maintained activity(from conversion to pyrazinamide), fail to confirm either of these characteristics(42).

Gram-negative Antibacterials.—1-Ethyl-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid(nalidixic acid) is one of two new active types reported in this decade. Its Gram-negative activity was first published by Lesher in 1962 and a brief structure-activity summary followed in 1964(43). There is no cross-resistance(44, 45) with other agents used in Gram-negative infections and its ED_{50} is comparable(46). The primary antibacterial action of nalidixic acid against E. coli is specific inhibition of DNA synthesis(47, 48). This antibacterial is unusual in being active as such and in rapidly forming an equally active metabolite, the 7-hydroxymethyl analog(49). About one-third is excreted as this metabolite plus two-thirds as glucuronides of parent and metabolite. Antibacterial activity(50) in urine is predominantly (7:1) due to 7-hydroxymethyl metabolite while in tissue the two agents are about equal in concentration(49). Clinical assessments(51, 52) rate it rapidly effective orally against certain Gram-negative bacteria. Since genitourinary tract infections due to these organisms should be treated for several weeks to avoid relapse, it is an important shortcoming that a high degree of resistance can develop rapidly during therapy(51, 53) or after a small number of transfers in vitro. A small trial(54) against brucellosis was successful. As is common in the early stages of clinical use, reports are conflicting on the breadth of application(55, 56, 57, 58) to urinary

infections (reported percentages of sensitive strains: 2-22%, 40%, 85%, 95%) and on side-reactions(51, 59).

Sulfanilamides.—Work on sulfa drugs is beginning its 4th decade with interest in and use of these agents continuing, especially since microbes apparently have not meanwhile progressively developed resistance(60). An application for sulfas (and other antibacterials) now receiving attention(61) is treatment of bacteriuria(frequently asymptomatic) of pregnant women, a condition with serious consequences for the child (higher incidence of prematurity, neonatal death, congenital abnormalities, cerebral palsy, and lower IQ) as well as for later chronic disease of the mother. The various drug-of-choice uses of sulfas have been summarized(62) and chemotherapeutic research with sulfanilamide derivatives reviewed(63). By means of pharmacokinetic equations and computers, various papers(64, 65) attempt to adjust experimental and clinical dosage of sulfas to get equitherapeutic plasma-water concentration-time curves.

Several long-acting sulfas introduced in recent years are in clinical evaluation (in approximate order of increasing persistence): 4-sulfa-3,6-dimethoxypyridazine(66), 3-sulfa-4-iodo-5-methylisoxazole(67), 2-sulfa-5-methoxypyrimidine(68), 2-sulfa-3-methoxypyrazine(69), and 4-sulfa-5,6-dimethoxypyrimidine(70). The latter is so slowly excreted that oral, parenteral or rectal doses once per week or per disease episode are being explored(71, 72, 73). In contrast to its 2,6-dimethoxy isomer, it yields only about 2% of glucuronide. In relation to crystalluria potential(68), the low solubility of the long-acting sulfas is partly compensated for by glucuronide formation, but more significant is the amount of sulfa and/or metabolite excreted per unit time in relation both to actual urine flow rate and to the therapeutic dosage required for the particular sulfanilamide.

Considerable study has been made of the binding of sulfas to blood albumin. Although bacterial inhibition varies directly with the percent unbound in a simple experimental model(74), the activity relationship in vivo can not be assumed to be the same as that of relative binding, and a highly bound compound need not be less desirable than a slightly bound one. Two agents with different degrees of binding to blood albumin also differ in binding to tissue, in vitro potency, pK_a, lipid solubility, excretion, metabolism, and distribution in body water, all of which directly or indirectly affect in vivo efficacy. In addition, the relationship of blood albumin binding to the degree of binding to the key enzyme site where the sulfa competes with PAB(75) is unknown. In an excellent symposium on interaction between drugs, Brodie(76) pointed out that binding is an advantageous and necessary characteristic without which a therapeutic agent would oscillate between toxic and inactive plasma concentrations and require frequent administration. Releasing an agent from binding to protein can be accomplished by means of a broadly similar chemical compound because of the relatively non-specific nature of the binding but this release can lead to greater toxicity(76) and excretion and metabolism as well as to the desired physiological action.

Of interest in laboratory and clinical studies, it has been observed that the mouse, dog, man and other species vary 17-fold in extent of albumin binding of sulfas. Variation of binding is 2-fold among normal human sera, and 10-fold in certain illnesses(77) partly due to circulating blood constituents and partly due to albumin variation. The locus of binding of several sulfas to serum albumin has been shown(78) by relaxation-time

measurements on high-resolution pmr spectra to involve the benzene ring and not the heterocycle; in 5-sulfa-1-phenylpyrazole the two benzene rings bind at different sites.

Information about sulfa drug metabolism in man has been reviewed(79). N^4-Acetylation has been known to occur enzymatically to an extent dependent on N^1-substituent and animal species. N^4-Glucuronides(1-4%) result from non-enzymatic chemical reaction while N^4-"sulfamates"(about 1%) are true metabolites. Formation of N^1-glucuronides occurs quite generally; with some N^1-heterocycles it amounts to only a few percent of dosage(sulfisoxazole, sulfathiazole) but with sulfamethoxypyridazine is substantial(15-20%) and with sulfadimethoxine is 60-85% of the substances excreted. 4-Sulfa-6-methoxy-2-methylpyrimidine yields a large amount of 2-hydroxymethyl O-glucuronide. Differences in the SO_2 infrared frequency in amino and imino isomers led to assignment of the sulfadimethoxine metabolite as N^1- rather than ring-N substituted(80, 81). 5-Sulfa-1-phenylpyrazole is 70-99% renally excreted as a glucuronide of still uncertain structure(82). 2-Sulfa-4,5-dimethyloxazole is reported(83) in a study by thin-layer chromatography to be grossly unstable in water as well as urine and blood, but a paper chromatographic study (84) flatly contradicts this.

Nitrofurans.--Twenty-two years after the first report of their antibacterial activity, this class is receiving renewed clinical and laboratory interest. In spite of several thousand publications covering some six hundred analogs and some structure-activity generalizations [required: 5-NO_2 group, furan ring, some kind of 2-substituent and lack of 3- or 4-substitution(85)], the critical characteristics that govern their activity especially in vivo are far from clearly defined. The chemotherapeutic properties of the earlier nitrofurans have been reviewed(86).

4-(5'-Nitro-2'-furyl)-2-(3''-pyridyl)thiazole is unusual in being a nitrofurylheterocycle bearing a heterocyclic substituent and in having broad-spectrum activity in vivo (about 8 times as active as furaltadone and furadantin). Unfortunately, blood dyscrasias occur at doses just below the therapeutic range(87). An experimental model was reported(88) for the polyneuropathy which occurs with furadantin and other nitrofurans(86, 89).

Recent work has involved linking nitrofuran to various heteroaromatic rings either directly or through one or more ethylene units. 3-(5'-Nitro-2'-furyl)-1,2,4- oxadiazoles and thiadiazoles and 2-(nitrofuryl)thiazoles were found low in activity in vitro and "largely ineffective" in animals (90) while the 1,3,4-isomers and 4-(nitrofuryl)thiazoles reported earlier were active in vivo (many at max. tol. doses). Hydroxymethyl- and bis-hydroxymethyl-amino derivatives(91) are less active and less toxic acutely than the parent known nitrofurylvinyl-aminoheterocycles. It is stated that in vitro activity is increased by insertion of vinyl or substituted vinyl between the 5-nitro-2-furyl unit and a pyridazine or thiadiazole(92), oxadiazole(93, 94), quinoline(95), pyridine(96) and its 1-oxide(97), range of minimal inhibitory concentrations being 0.03 to 10mcg/ml. The insertion of another vinyl group is claimed to increase the in vitro activity still further for oxadiazoles(98) and pyrimidines(99), range of m.i.c. 0.02 to 0.3mcg/ml. Several were found active in vivo but only at maximum tolerated doses. 3-(beta-Substituted ethyl)-1-(5'-nitro-2'-furfurylideneamino)-imidazolidin-2-ones are claimed(100) to be more active in vivo but less active in vitro than the 3-H parent(101).

Antileprotic Agents.—Although the age of chemotherapy has benefitted its treatment, the effect on this wide-spread(102) disease is incomplete and slow. The slowness is befitting this, the most ancient infectious disease of man, which has the longest known incubation period (from 3 to 8 or more years) and the longest generation time (30 days)(102, 103). New agents have come from trying in human leprosy anything active against tuberculosis, hoping for activity on the basis of the taxonomic relationship of the causative organisms. Neither an in vitro test nor an experimental form of the disease has been available but recent advances have been made(102, 103, 104, 105). Although some antituberculotics are effective against human leprosy, activity against tuberculosis is an unreliable indicator(106) of human antileprotic activity, and vice versa.

Three reports of activity have come from clinical use of agents available for widely differing purposes. Thalidomide(α-phthalimidoglutarimide), while being employed as a sedative, showed activity against human lepromatous leprosy in 6 consecutive cases by several criteria. It will be interesting to await confirmation of this preliminary report(107). In another preliminary report(108) involving a more extensive trial(50 patients) in a controlled comparison with diaminodiphenylsulfone, ethambutol was reported equally active. Against an experimental Myco. lepraemurium infection(109) in mice it was estimated to be somewhat less active than the sulfone. More active than this standard in the murine infection are 4-sulfa-6-methoxypyrimidine and 3-sulfa-6-methoxypyridazine. Several papers on clinical efficacy of the latter have appeared(110, 111). Using the murine test, 1-[3-(4-substituted phenyl or pyridyl)-5,6,7,8-tetrahydro-1-naphthylaminopropyl] piperidines were found to have an interesting level of activity(112).

In Vitro Actives.—Such compounds are potentially useful for bacterial control of body cavities, genitourinary tract, wounds, skin prior to operations, hands of medical personnel, and for preservation of parenteral and topical medicinals. The significance of many publications can not be judged since tests are not adequately defined or related to standards nor are toxicity, in vivo results and effect of protein on activity given. The following were active against Gram-positive and Gram-negative organisms at the minimal inhibitory concentrations given: 6-(4'-methoxyphenyl)-imidazo[2,1-b] thiazole(113) at 8-60, 2-(phenyl and 2'-pyridyl)-isatogen(114) at 25-200, 1-(n-dodecyl)-2-(dichloroacetyl)guanidine(115) at 0.05-25, and 3-(methyl and ethyl)-1-hydroxy-2-oxoquinoline(116) at 0.3-5mcg/ml. In the latter which is 1-10 times as active as aspergillic acid, the hydroxamic acid moiety is necessary but not sufficient for activity. Dibenziodolium(117, 118) and dibenzoxaiodinium analogs(119) were claimed to be more stable and more active (m.i.c. 0.3-25mcg/ml) than the diphenyliodonium analogs; acute LD_{50} 10-100mg/kg.

Work continues on detergent bases and cationic surfactants, whose activity was first reported in 1935. Antibacterials of this kind generally inhibit both Gram-positive and Gram-negative organisms. 2-(N-Decylpyrrolidinium) ethyl trans-beta-(1-naphthyl)acrylate(120) was 50-100-fold less active in the presence of serum, an effect common to many agents of this class. Other compounds active were polychloro-2,3-diphenylpropylamines(121) and 3,3,3-tris(4'-chlorophenyl)propyl-N,N-bis(beta-pyridinium ethyl)amine dichloride at 1-100mcg/ml. Several kinds of phenylenebis (4-amino-6-amidinoquinaldines (122) were active at the same concentration but were not active in vivo. The activity of N-(3-phenyl-2-propyl)-3,3-diphenylpropylamine(123) at 1-10mcg/ml

against Gram-positive organisms is reversed by nucleic acids or by divalent cations. The latter also antagonized the action of steroidal tertiary amines or quaternaries(124) whose cellular damage is prevented by spermine also.

5-Azacytidine at 0.2mcg/ml inhibits growth of E. coli as a result of spontaneous triazine ring-opening in the nucleic acid into which it is incorporated(125). Mono and di-chloro 2-benzamido-4,6-dialkoxypyrimidines(126) inhibited Staph. aureus at 1-5mcg/ml but the somewhat more active 4-sulfa-2, 6-dimethoxypyrimidine standard inhibited Gram-negative organisms also. Their activity may be a variant of the halo-salicylanilide type(127). 2,4-Dialkanoyl phloroglucinols(128) also have narrow spectra(Staph. and Strep. inhibition at 0.1-0.5 mcg/ml); related resacetophenones are inactive(129). A relatively unstable chelate permits cell penetration by salicylaldehydes whose activity against Pseud. aeruginosa is dependent on the amount of free aldehyde group liberated inside the cells(129). This organism is subject to bactericidal action (130) by ethylenediaminetetraacetic acid but not other chelators; it produces damage to the protoplast membrane.

Research Techniques and Screening Methods.—Radioactive tracers have been used in studying pharmacology and mechanism of action. The primary antibacterial action of nalidixic acid was shown(47, 48) to be specific inhibition of DNA synthesis by following the incorporation of ^{14}C- and ^{3}H- labeled purines, pyrimidines and amino acids into DNA, RNA and protein of E. coli. Ethionamide's mechanism of action was investigated(38) by means of ^{32}P-, ^{35}S- and ^{14}C- labeled nucleic acid and protein precursors. ^{14}C-Ethambutol was employed in human pharmacological(20) and mechanism of action(19) studies. ^{14}C-Labeled quinaldinium compounds were used to determine their mechanism of antibacterial action(131).

Pharmacokinetic equations are being solved by analog computers and used (64, 65, 132, 133) to devise optimal dosage regimens which are important to safe, effective clinical trials and can make laboratory comparisons more incisive by equalizing exposure of infections to drugs. These equations have been applied in a series of papers to sulfa drugs(64, 65, 133) for which extensive pK_a, protein binding, excretion, and distribution data are available.

Adequate screening methods for leprosy have been lacking but tissue-culture(102) and in vitro growth(105) have been disclosed. Growth of the human strain in cell-free medium has resulted(105) from use of unisolated growth factors from lysed saprophytic mycobacteria. One method available has been the mouse foot-pad growth(103) of Myco. leprae which does not produce a leprosy infection and might be considered a form of tissue-culture. A Myco. lepraemurium infection(109) in mice has several similarities to the human disease but also certain differences. According to a recent report(104), the infection of black mice with "Chatterjee" bacilli is not the hoped-for major step forward in antileprotic screening but seems to be a modified M. lepraemurium infection.

A lethal TB infection was developed(134) in guinea pigs by intracardial injection of M. tuberculosis H37Rv but has a more variable survival time than the mouse infection and requires more compound for testing. Various kinds of delayed and intermittent treatment of tuberculous mice have been studied(135) with half a dozen reference drugs.

Correlations of molecular vibrations, π-electron energies and of calculated molecular orbital and bond-hybridization character with antibacterial activity(136, 137, 138, 139) provide interesting summaries of where one has

been rather than where one is going. Perhaps when we know how to transfer in vitro data successfully to in vivo tests, we will be able to make use of such specialized, "unidirectional" theoretical approaches.

Modern physical methods and chromatographic techniques are being applied to problems of separation and identification of synthetic antibacterial structures and their metabolites, which in the case of nalidixic acid makes a major contribution to activity: gas chromatography (14, 140); thin-layer chromatography of sulfanilamides(141, 142), amines(143), and heterocycles (144); mass spectrometry of pyrimidines(145) and furans(146); nmr spectrometry of phenols(147, 148), peptides(149), and pyrimidines(150); X-ray spectrometry of P-, S-, and halogen- containing compounds(151); and gamma-ray spectrometry of neutron-activated pharmaceuticals(152) employed in forensic investigations but broader in potential(153).

Mechanism Of Action.—The enzymic bases of now well-defined mechanisms for bacterial control (competitive inhibition of essential enzymes, feed-back inhibition of biosynthesis of the natural competitor, repression of formation of a necessary enzyme, and non-competitive inhibition by reacting with the enzyme active center) have been reviewed(154). The biochemical basis for differential action of an antibacterial toward host and microorganism is clear for a number of synthetic agents(156). Differences in response to dihydrofolate reductase inhibitors reflect differences in the structural limitations of the binding site of the same enzyme from different bacteria(155).

Sulfanilamides are unique in having their mode of action almost completely known at the enzyme level. The step in folic acid synthesis at which they intervene has recently been shown to be the reaction of PAB with the pyrophosphate ester of 2-amino-4-oxo-6-hydroxymethyldihydropteridine(155). The sulfas are now known to participate in this reaction and form spurious folic acids, but their significance in growth inhibition is not certain. Some differences in responses of bacteria are due to relative abilities to transport and assimilate folic acid. Sulfanilic acid is an inhibitor of a cell-free enzyme system but not of bacterial growth, presumably due to difficulty with cellular transport(155).

Bactericidal action of sodium tetrapropylenebenzenesulfonate resulted from lysis of protoplast membranes; in contrast to many other agents, this action decreased as cellular metabolic activity increased(156). Sodium lauryl sulfate produces inhibition of biosynthesis of S. aureus cell walls similar to that produced by penicillin(157).

Prevention of emergence in vitro of drug resistance to a variety of antibacterials by spermine or atabrine was reported(158) to result from preventing induction of resistance. An antimutagenic action of spermine toward random as well as induced mutations(159) confirms the effect (stated not to be co-inhibition) in vitro but demonstration of a useful effect in vivo is still lacking.

Reviews.—Reviews appeared on tuberculosis(15, 29, 160, 161), leprosy(106), sulfa drugs(63, 64, 65, 77, 79, 133), nitrofurans(86), drug interaction(76), protein binding(77, 133), mechanisms of action(154, 155, 162), and combination therapy(163).

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Chapter 13. Antiviral Agents

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Introduction

In some measure, perhaps, late 1964 was a turning point in the pursuit of antiviral drugs. In December of that year a conference of the New York Academy of Sciences summarized in detail the progress made in antiviral research. Reference to the proceedings[1] of this conference, published in July 1965, will conveniently bring one up-to-date to the beginning of 1965. The considerable interest in antiviral research today appears to be, understandably, in direct proportion to those clinical successes achieved in the last few years. No longer is this an area only of academic interest; there is little question that drugs can safely alter the course of human viral disease.

The following review is largely a summary of the continuing activity induced by those drugs shown to have an antiviral effect in man. No attempt will be made to summarize the substantial literature on interferon, as worthy an antiviral agent as it may be. Also during 1965 two review articles were published on antiviral agents[2,3] the one by O'Sullivan being of special interest to the medicinal chemist.

Thiosemicarbazones

In 1962 Bauer[4] began field trials which eventually produced convincing evidence that methylisatin-β-thiosemicarbazone (also known as Marboran, methisazone, and compound 33T57) effectively aborted smallpox among 5,000 household contacts during an epidemic in Madras, India. Additional studies indicate that this compound may have some clinical usefulness in progressive generalized vaccinia (vaccinia gangrenosa)[5] and generalized vaccinia in eczematous patients (eczema vaccinatum).[6] Some newer derivatives of thiosemicarbazones have been found to produce a considerable degree of protection in mice infected with neurovaccinia virus.[7] Jones and co-workers[8] reported that 4-formylpyridine-thiosemicarbazone has antivaccinia activity only when administered orally; this suggests that the drug is converted to an active antiviral substance in vivo.

Studies with ethylisatin-β-thiosemicarbazone indicate that it achieves antiviral activity in mice by reducing the multiplication of virus by 99%.[9] According to Woodson and Joklik,[10] isatin-β-thiosemicarbazone has no effect on the synthesis of vaccinia virus DNA (deoxyribonucleic acid) but it causes a reduction in the number of polyribosomes formed from the mRNA (messenger ribonucleic acid) necessary for virus protein synthesis. There has been a report that certain thiosemicarbazones may have activity against influenza A virus (PR-8) in mice.[11]

Nucleosides

The observation by Kaufman of the efficacy of 5-iodo-2'-deoxyuridine (idoxuridine, IDU, or IUdR) for the treatment of herpes simplex virus infection of the human eye has been confirmed and reviewed by Leopold[12] IDU, which is an analog of thymidine and a specific inhibitor of DNA-containing viruses, has been licensed for use in the United States and is now available to the physician.

Thomas and co-workers[13] and Hart and co-workers[14] have reported favorable results with IDU in the treatment of superficial dendritic keratitis. In those infections involving the deeper stromal layers of the eye, treatment with IDU seemed to be effective when combined with corticosteroids,[15] chymotrypsin,[16] or antibiotics.[15] Presently the accepted regimen for IDU treatment involves application of the drug to the eye every hour through the day and every 2 hours at night. Shell[17] reported that this treatment schedule is inferior to the regimen of one drop of IDU solution applied to the eye every minute for 10 minutes, repeated four times a day.

There has always been some concern about the toxicity of a drug that so closely resembles a constituent of DNA. So far there are no reports clearly indicating toxicity of IDU in the eyes of man or animals. One might still suggest that such an agent could have a mutigenic effect. Rothstein and Weinsieder[18] reported that IDU was incorporated in the lens of experimentally injured rabbit eyes at the same rate as thymidine. Hanna and Wilkinson,[19] however, found that IDU applied to normal or mechanically injured rabbit corneas did not influence the incorporation of tritiated thymidine into cellular DNA. These authors further pointed out that IDU has selective inhibitory action on the multiplication of herpes simplex virus in the eye without altering DNA synthesis or multiplication of corneal cells.[19]

It is not clear why a more vigorous attempt has not been made to apply IDU and similar inhibitors of herpes simplex virus to herpetic skin lesions. No single virus serotype produces more disease in man than herpes simplex virus, yet there have been but a few drug studies and these were poorly controlled. An injection of IDU intradermally with a "Mark 2" air gun (Dermojet) was reported to produce marked improvement in dermal herpetic infections in a double-blind human trial, while topical application of IDU ointment appeared to have no beneficial effect.[20] The drug has been reported to inhibit other DNA viruses, including polyoma,[21] myxoma, fibroma,[22] and bovine rhinotracheitis in tissue culture.[23] There is also a report of IDU influencing the course of the RNA virus of encephalomyocarditis in mice;[24] however, in previous publications this seemed to be more an effect on the animal than on the virus.[25] IDU apparently had little influence on herpes B virus infection of rabbit eyes.[26]

It has been suggested that resistance of certain strains of herpes simplex virus to IDU could be explained as due to inability of the virus to induce thymidine kinase synthesis in host cells; however, a report by Centifanto and Kaufman[27] suggests this is not true. Both IDU and 5-bromo-2'-deoxyuridine (BDU) are used to determine, by indirect means, the nucleic acid content of viruses. The recent demonstration, for example, that although BDU inhibits DNA viruses it had no effect on Coxsackie A-21 and rubella viruses, indicates that these two viruses are RNA-containing viruses.[28] IDU and BDU do not always completely free a cell culture system of the DNA virus; however, if used in combination with such antivaccinia virus drugs as noformicin, 5-methyl-tryptophane, or IBT, cell cultures could be completely freed of vaccinia virus.[29]

Recently a number of other nucleosides have been reported to have inhibitory activity against DNA viruses including iodouracil arabinoside, thymidine arabinoside, adenine arabinoside,[30] deoxyadenosine,[31] and 5-methylamino-2'-deoxyuridine.[32] It was found that this last-named compound,[32] as well as a soluble protein fraction extracted from a broth culture of phage-infected Escherichia coli,[33] would cure rabbit's eyes of herpes simplex virus infection. There is still an additional report that urgocyton, a noncoagulable protein of unrevealed origin, apparently had beneficial effects on eye infections in

man that were associated with the DNA-containing herpes zoster virus when the compound was administered intramuscularly in doses of 5 mg per day for 7 days.[34] This material also was reported to suppress vaccinia virus skin lesions in animals.[34]

There have been no recent reports on the usefulness of cytosine arabinoside (cytarabine) against herpes simplex virus eye infections, probably because some drug toxicity for the cornea has been detected in the past.

35 Amantadine

Jackson and co-workers previously established the 1-adamantanamine hydrochloride (1-AH, 1-aminoadamantane, amantadine) influenced the course of influenza A2 virus infection in man. Recent reports indicate that, in tissue culture or eggs, 1-AH suppresses influenza A_0 (PR-8, Swine, WS, NWS), A_1 (FM1), A_2 (Jap/305, Jp^c, AA Pak/1/57), C (1233), parainfluenza type 1 (Sendai), and rubella, but not influenza B (GL, Lee).[36-39] The antiviral agent appears to act by reducing virus penetration into susceptible host cells.[40]

When 1-AH was administered by the intraperitoneal, oral, or intranasal route, mice were protected from lung infections caused by influenza A (Swine) and A_2 (AA) viruses.[41] Other reports indicate that this compound is ineffective against certain oncogenic viruses in mice[42] and rubella virus in monkeys.[43] Davies and co-workers[44] were able to reduce multiplication of influenza virus in mouse lung up to 99.9% with 1-AH treatment following a relatively low dose of virus, and the survivors were immune to rechallenge with up to 100 times a 50% lethal dose of the same virus. The drug was reported to be rapidly absorbed in mice when it was given by the oral route, concentrated in the lung tissue, and rapidly excreted in the urine of both animals and man.[45] There is no evidence that 1-AH is metabolized by man after oral administration.[45]

A number of amantadine derivatives have been tested against influenza A viruses in tissue cultures and animals.[46] According to Tsunoda and co-workers[47] α-methyl-1-adamantane-HCl has a higher protective index against influenza A_2 (Jap/305) virus in tissue culture than 1-AH, as well as increased potency against rubella and rubeola virus infections of tissue cultures.

Another compound long claimed to be active against influenza, N'N'-anhydrobis-(B-hydroxyethyl)-biguanide-hydrochloride (ABOB, Flumidin) has recently been reported to show a "weakly" protective effect in influenza virus infections of man.[48]

Newer Antiviral Agents

Largely because of the interesting results produced by the compounds just discussed, there appears to be a greater effort in searching for other viral inhibitors as reflected in the increased number of reports of such agents from antibiotic filtrates, plant extracts, and organic chemicals. The following discussion will briefly review these reports, none of which contain any clear implications in clinical medicine.

Lumb and co-workers[49] isolated two antiviral antibiotics, borrelidin and vivomycin, from a Streptomyces species. Vivomycin was so named because it was active only in vivo against encephalomyocarditis (EMC) and influenza viruses.[49-50] Borrelidin is a macrolide antibiotic which seems to act synergistically with vivomycin in mice infected with EMC virus.[50,51] Both vivomycin[50] and statolon,[52] a product of a penicillium mold, seem to enhance host defense mechanisms and perhaps induce interferon formation. Dales[53] reported that the

antibiotic streptovitacin A inhibits vaccinia virus by suppressing specific enzyme synthesis required for the uncoating of intracellular virus. Distamycin A apparently suppresses multiplication of DNA viruses in tissue culture and herpetic eye infections in rabbits.[54] Rutilantin, an antibiotic from an actinomycetes, seems to inhibit some step in the multiplication cycle of rabbitpox virus in tissue culture.[55]

Tannic acid, flavonols,[56] and caffeic acid[57] are plant-derived substances that readily inactivate herpes simplex virus on contact while the plant growth regulator 2,3,5-triiodobenzoic acid appeared to suppress tobacco mosaic virus infectivity of tobacco leaves.[58]

Hydroxybenzylbenzimidazole has long been known as an inhibitor of certain enteroviruses in tissue culture. It was reported that the methoxy and hydroxy methyl derivatives are more active than the parent compound in suppressing RNA synthesis of poliovirus.[59] Certain thiopyrimidine derivatives[60] and biguanides[61] have been observed to inhibit multiplication of some myxoviruses.

The antibiotic amicetin[62] and 2,4-dioxo-5'-thiozolidine acetic acid derivatives[63] inhibit herpes simplex virus, whereas 6-aminonicotinamide suppresses multiplication of vaccinia virus but not herpes simplex virus.[64] Inglot and co-workers[65] reported that p-nitrobenzylphosphoric acid inhibits EMC virus in tissue culture systems but has limited activity against the same virus in animals. Kochman and co-workers[66] investigated structure-activity relationships of organic compounds related to salicylic acid and certain phosphoric and carboxylic acids for antiviral activity against EMC virus in tissue culture. Their studies revealed that dibenzyl compounds were more potent than monobenzyl derivatives and that presence of a nitro group increased the antiviral activity while amino groups decreased it. Sidwell[67] observed that 1,3-bis-(2-chloroethyl)-1-nitrosourea saved mice that were infected with leukemic viruses. Another report indicates that N-hydroxy-methane inhibits Shope fibroma virus in tissue culture but is only slightly active in rabbits.[68]

Summary

Pertinent publications on the subject of antiviral drugs for 1965 have confirmed and extended previous clinical observations of the activity of N-methyl-isatin-B-thiosemicarbazone (Marboran) against smallpox, 5-iodo-2'-deoxyuridine (IDU) against herpes simplex keratitis, and 1-adamantanamine hydrochloride (amantadine) against influenza A virus infection. Many newer antiviral substances, both natural and synthetic, have been found to be active in tissue culture systems and animals but as yet have not been studied in human viral infections.

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Chapter 14a. Human Antiparasitic Agents

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I. PERSPECTIVES

The great advances made in chemotherapy during this century have enabled some measure of control of the major killing diseases of the tropics. Notwithstanding past progress, man today is confronted by a catalog of challenging problems, and the incentive to develop novel and improved antiparasitic agents has not diminished. Adequate drugs are still lacking for the treatment of many chronic and debilitating diseases including leishmaniasis, Chagas' disease, filariasis, schistosomiasis, clonorchiasis, trichuriasis, and strongyloidiasis. Moreover, reports of drug resistance have often followed advances in chemotherapy like a "faithful shadow", and this shadow will no doubt lengthen in the future.

The philosophy of global eradication of communicable diseases[1] and the evolution of techniques for its achievement have added a new dimension to research in parasite chemotherapy, namely the urgent need for drugs with protracted action. While the clinician interested in treating an individual often has a variety of good drugs at his disposal and may feel little need for new agents, the public health worker, whose aim is the ultimate eradication of a disease from an entire community, faces problems of a magnitude and type not encountered by the clinician and is frequently unable to achieve his objective of total coverage with currently available drugs.

Future progress in parasite chemotherapy will depend mainly on an increased awareness of such critical problems and needs and on appropriate recognition of biochemical processes within both parasite and host. Intensive efforts to develop useful agents for the treatment of parasitic diseases of livestock, poultry, and other domestic animals have also yielded a variety of promising new substances (see Chapter 14b), and the benefits derived from interplay between human and veterinary research are already apparent.

II. MALARIA

In spite of the impressive gains made in global malaria eradication, malaria still disables more people and imposes a heavier economic burden than any other disease. Today more than one billion people live in malarious regions: 723 million in areas where eradication programs are in progress and 393 million in areas where programs have not yet started.[2]

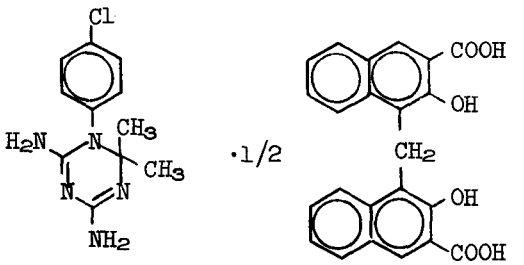
The ready availability of a variety of useful synthetic antimalarial drugs in the post-World War II era contributed to a feeling that malaria would soon cease to be a world problem, and interest in the development of

new drugs waned. Moreover, only recently has the potential usefulness of chemotherapy in all phases of malaria eradication been fully recognized.[3] A reappraisal indicated: (1) the need for long-acting drugs in malaria eradication programs;[3-5] (2) the seriousness of the situation created by the possibility of widespread resistance of Plasmodium falciparum to the 4-aminoquinolines and the resulting urgent need for new types of fast-acting suppressive drugs;[6,7] and (3) the importance of developing safe and effective anti-relapse drugs capable of effecting a radical cure of P. vivax and P. malariae, preferably in a single dose or at most in a three-day regimen.[3]

Several excellent, authoritative reviews on malaria chemotherapy have recently been published.[3,8,9]

A. Repository Drugs. - The development of repository antimalarial drugs required the synthesis of substances that would prolong the release of active moieties from depot sites or, like 6,8-dichloro-2-phenyl-α-2-piperidyl-4-quinolinemethanol (SN-10,275), be firmly bound by host tissues and then slowly released.[10]

1. Dihydrotriazine and Pyrimethamine Salts. - The synthesis of cycloguanil (DHT) pamoate (Camolar®) (I)[10] and other dihydrotriazine[10,11] and pyrimethamine[10,12] salts that exhibit remarkable repository antimalarial properties[5,10] has been reported. A single intramuscular dose of cycloguanil pamoate has the unusual capacity to protect man for many months against challenges with susceptible strains of Plasmodium vivax,[13,14] P. falciparum,[15,16] P. malariae,[16] and P. ovale.[16] Although cycloguanil pamoate has so far not shown a liability to induce rapid resistance, parasites known to be resistant to chlorguanide, DHT, or pyrimethamine are less susceptible to it.[14,17-19]



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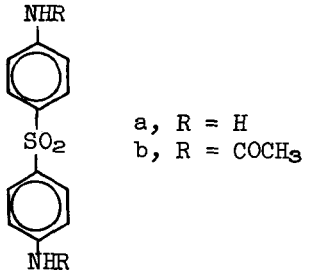


a, R = H
b, R = $COCH_3$

II

2. Sulfones and Sulfonamides. - The sulfones and sulfonamides (vide infra) act at a different site than DHT or pyrimethamine, presumably by preventing the incorporation of p-aminobenzoic acid into folic acid.[20] Potential long-acting sulfone and sulfonamide derivatives were synthesized to provide substances that, in combination with cycloguanil pamoate or related compounds,[11,12] might enable a sequential block in the metabolic synthesis of nucleotides and afford broader repository action against drug-resistant lines than either drug alone.[21] Various 4-acylaminodiphenylsulfones exhibited

promising repository action in mice. Against trophozoite-induced P. berghei infections in mice, a single subcutaneous 100-400 mg./kg. dose of N,N'-diacetyl-4,4'-diaminodiphenylsulfone (DADDS) (IIb) almost invariably prevented or strongly suppressed patent infections through 6 to 14 weeks. A single 50 mg./kg. intramuscular dose of DADDS prevented patent P. cynomolgi infections in monkeys for 63-268 (average 158) days and greatly suppressed the parasitemia for many weeks longer. A comparison of DADDS, cycloguanil pamoate, and a 1:1 mixture against lines of P. berghei highly resistant to either DDS or DHT demonstrated that the mixture had broader repository action against the drug-resistant lines than either drug alone.[21,22] Further evaluation of the DADDS-cycloguanil pamoate mixture in connection with the prevention and eradication of malaria is in progress.

3. 4-Aminoquinolines. - Several novel 4-aminoquinolines have also been tested for therapeutic and repository activity against trophozoite-induced P. berghei in mice.[23,24] The most interesting of these, 4,4'-[1,4-piperazinediylbis(1-methylethyleneimino)]bis[7-chloroquinoline] (III), protected mice against intervening challenge with P. berghei for 8 weeks following a single oral dose of 500 mg./kg. The compound has a marked affinity for liver and kidney, and a delay in the release of the drug from liver parenchymal and Kupffer cells is apparently responsible for the striking repository effects. No reports have yet been published to indicate whether or not the drug has significant repository action in other laboratory animals or in man. The synthesis of III and many related compounds has been reported.[25]

4. Silicone Rubber Implants. - Silastic capsules have been used successfully in experimental animals for the sustained release of antimalarial and antischistosomal agents.[26]

B. Suppressive Agents. - From the dawn of chemotherapy progress has been shackled by the appearance of drug resistance. Sporadic observations of lessened sensitivity of malarial parasites to quinine, quinacrine, pamaquine, chlorguanide, and pyrimethamine appeared prior to 1960, but the impact of this phenomenon on malaria eradication was considered insignificant.[7] Recent investigations indicate that certain strains of P. falciparum from South America and Southeast Asia are resistant to the 4-aminoquinolines - the most widely used drugs in malaria chemotherapy - and that some of these strains are also less susceptible to many, or in a few instances all, widely used antimalarial agents.[7,8,27-29] The possible impact of these findings on the prevention, treatment, and eradication of malaria is obvious and underscores the need for new antimalarial drugs.[3,6-8,27,30]

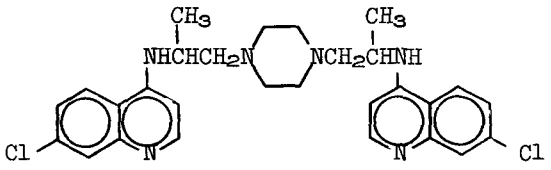
1. Sulfones and Sulfonamides. - It has been known for several decades that sulfones and sulfonamides have antimalarial action, but none has been used extensively in the treatment of human malarias.[3,8,21] Recent observations, however, have revived interest in the possible utility of these substances both as repository (vide supra)[21,22] and suppressive[29-35] agents.

Certain lines of P. berghei, P. cynomolgi, and P. gallinaceum made resistant to 4,4'-diaminodiphenylsulfone (diaphenylsulfone, DDS) (IIa) or to DHT or pyrimethamine were still susceptible to the heterologous drug with only a low order of cross-resistance.[31] Further, a 1:1 mixture of DHT and DDS proved

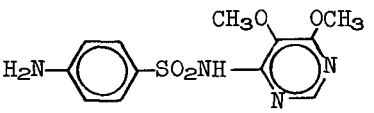
highly effective against the parent, DHT-resistant, and DDS-resistant lines of P. berghei, and the rate of emergence of resistance in the parent strain was significantly less with the mixture than with either drug alone.[31] In man daily oral doses of 25 to 50 mg. of DDS protected 22 of 26 volunteers from infection by strains of resistant P. falciparum isolated in Southeast Asia.[30]

Pyrimethamine (50 mg. daily for 3 days) or sulfadiazine (2.0 g. daily for 5 days) given alone did not cure infections with the Malaya (Camp.) strain of chloroquine-resistant P. falciparum; these doses of pyrimethamine and sulfadiazine administered concurrently, although not rapidly effective, cured infections in 5 of 6 volunteers.[29] Sulforthomidine (Fanasil®) (IV) given weekly in 500 mg. doses alone or in combination with 25 mg. of pyrimethamine cleared symptomless asexual parasitemia in East African schoolchildren infected with pyrimethamine-resistant P. falciparum.[32] Although this study indicated that sulforthomidine acted too slowly for use against acute attacks, 25 semi-immune patients were subsequently treated with single 1.0 g. doses, and all but 3 were cured within 3 days.[33] Sulforthomidine appears to have no sporontocidal action against P. falciparum.[34]

The above findings, together with indications that the combined oral use of a sulfone or sulfonamide with pyrimethamine or chlorguanide may lead to potentiation of antimalarial effects,[8,24,31,35] pinpoint an area worthy of extensive investigative efforts.



III

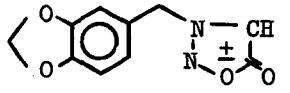


IV

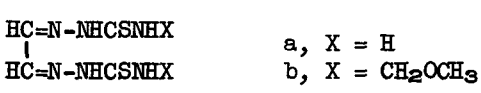
2. 4-Aminoquinolines. - In therapeutic studies against a normal P. berghei strain in mice, III had an oral CD_{50} of 10 mg./kg. daily x 4.[23] Although chloroquine-resistant strains studied to date have proved uniformly resistant to other 4-aminoquinolines,[8] a chloroquine-resistant strain of P. berghei surprisingly was reported not to be cross-resistant to III.[24]

3. New Structures. - Several new active chemical moieties have been reported. 3-Piperonylsydnone (V) was active against P. berghei at a dose of 10 mg./kg.[36] The glyoxal dithiosemicarbazones VIa and b showed high activity against P. gallinaceum in the chick; the median effective doses per os were 0.7 mg./kg. x 7 and 0.5 mg./kg. x 7, respectively.[37] It is not yet clear whether these or related compounds will have practical application.

C. Radical Curative Agents. - The 8-aminoquinolines are the only substances having sufficient activity against secondary tissue schizonts to be of practical value in achieving radical cure of relapsing malarias. However, primaquine and certain other 8-aminoquinolines produce acute hemolytic anemia



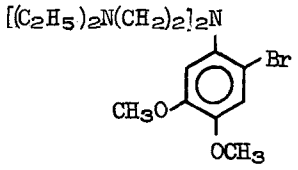
V



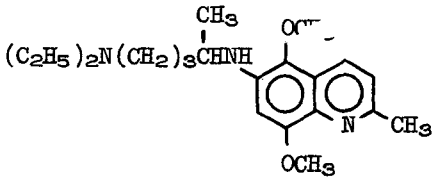
VI

in persons whose red cells are unusally susceptible to drug-induced hemolysis. The rapid degradation and elimination of primaquine necessitates daily administration, which constitutes a further drawback limiting its use.

The discovery that 4-(2-bromo-4,5-dimethoxyphenyl)-1,1,7,7-tetraethyldiethylenetriamine (RC-12) (VII) has a pronounced effect on the exo-erythrocytic stages of P. cathemerium in canaries[3] stimulated an appraisal of the activities of RC-12 against P. cynomolgi in the monkey.[38] Although the drug per se had little promise as a schizonticidal or suppressive drug, it showed significant promise as a prophylactic or radical curative agent and might find use when: (1) the combination of chloroquine-primaquine is not effective in causal prophylaxis, as appears to be the case where there is chloroquine resistance; (2) there is a need for a curative agent which can produce benefits in less than 14 days; and (3) there are fears of enhanced susceptibility to the hematotoxicity of primaquine. Clinical studies are planned.[38]



VII



VIII

6-[[4-(Diethylamino)-1-methylbutyl]amino]-5,8-dimethoxyquinaldine (B-505) (VIII)[39] also produced radical cures of P. cathemerium infections in canaries, but the relative toxicity of this compound precluded clinical studies in man.[3]

D. Test Methods. - The demonstration of multiple drug resistance in P. falciparum (vide supra) spurred research to develop better experimental models for studying this phenomenon. Strains of P. berghei have been induced to acquire resistance to representatives of all the major classes of suppressive drugs, and the resistant lines have been employed in cross-resistance tests to determine the interrelationship among the various chemical types,[20,31,40-44] Anopheles stephensi has been shown to be a suitable experimental vector of P. berghei to serve as a source of sporozoites and for cyclic transmissions, and the existence of primary tissue stages of P. berghei has been confirmed.[45,46] The relative ease of infecting the splenectomized gibbon with blood-induced infections of P. falciparum indicates that this species may be a suitable laboratory model for studies of falciparum malaria.[47]

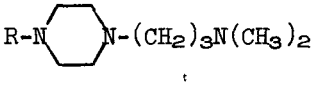
III. LEISHMANIASIS

Leishmania is responsible for millions of smoldering ulcers among inhabitants of endemic regions in Africa, the Americas, and the Near and Far East. Antimonials are usually effective in leishmaniasis, but the necessity for repeated (10-30) injections and the well-known contraindications of these drugs impose serious restrictions on their widespread use. Inasmuch as some regression of early lesions of dermal leishmaniasis was observed in malaria patients treated with chlorguanide,[48] it was of interest to investigate the potential usefulness of the repository drug cycloguanil pamoate (I) (vide supra). Early results from Costa Rica and Mexico are encouraging; a single intramuscular injection of cycloguanil pamoate (280-350 mg. base) cured 26 of 30 patients with cutaneous and mucocutaneous infections of L. braziliensis[48] and 31 of 40 persons with cutaneous leishmaniasis produced by L. mexicana.[49]

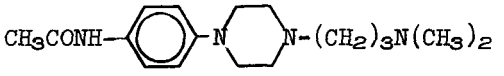
IV. CHAGAS' DISEASE

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 success attending the development of effective drugs for the African trypanosomiases, the treatment of cruzi infections has been generally unsatisfactory. There is some evidence that nitrofurans may be effective against acute infections, but no curative agent has yet been found.[50]

The discovery that N^1-(3-dimethylaminopropyl)piperazines (IX) exhibit strong chemotherapeutic activity against T. cruzi in mice is of particular interest.[51] The nature of the R substituent is relatively non-specific, but modifications of the N^1-(3-dimethylaminopropyl)piperazine moiety drastically



IX



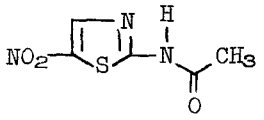
X

reduce activity. In comparative studies with T. cruzi in mice, piperamide maleate (X), given orally in sixteen 2.5 or 10 mg./kg. doses over 7 days, prolonged the median survival time of treated mice significantly longer than mice treated with these doses of furaltadone or primaquine.[51] However, the curative value and the safety of piperamide maleate and related substances[52] have yet to be established.

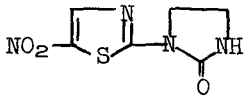
V. AMEBIASIS

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 satisfactory antiamebic agents are available, no single preparation is capable of exerting all the actions required. Therefore, the search for new agents continues.[53]

A. 2-Amino-5-nitrothiazoles. - Over a decade ago it was reported that 2-acetamido-5-nitrothiazole (aminitrozole) (XI) possessed curative action against intestinal amebiasis in rats and dogs and killed adult Schistosoma mansoni in mice at well-tolerated doses.[54] More recently a new 2-amino-5-nitrothiazole derivative (Ambilhar®, 32,644-Ba) (XII) having strong therapeutic effects against amebiasis and schistosomiasis (vide infra) was discovered.[55] The minimum inhibitory concentration of 32,644-Ba against E. histolytica in vitro was 10 μg./ml. This is approximately the same potency exhibited by emetine or 4,7-phenanthroline-5,6-quinone under comparable conditions.[56] The compound strongly suppresses amebic liver abscess in hamsters following daily oral doses of 60-120 mg./kg. for 7 days (chloroquine was effective at 100-200 mg./kg.). Intestinal amebic infections in rats and guinea-pigs were markedly reduced following daily oral doses of 30-60 mg./kg. for 4 and 5 days, respectively.[56] Results of preliminary human trials indicate that 32,644-Ba, administered in daily doses of 20-25 mg./kg. for 5 to 10 days, is effective against both amebic dysentery and amebic liver abscess, although side effects including frequent ECG changes and occasional neuropsychic episodes were noted.[57] More extensive trials will be required to assess the relative safety and efficacy of the drug compared with other forms of treatment. Related heterocyclic compounds are also reported to have antiamebic and antischistosomal properties.[58]

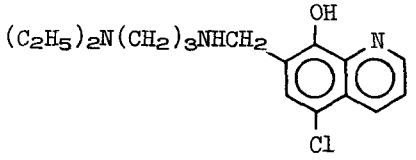


XI

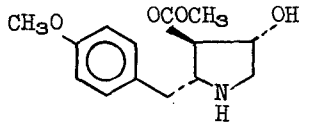


XII

B. 8-Quinolinols. - Another new amebicide of potential interest is 5-chloro-7-(3-diethylaminopropylaminomethyl)-8-quinolinol (clamoxyquin, PAA-3854, CI-433) (XIII).[59] The relative amebicidal concentrations of clamoxyquin hydrochloride and emetine hydrochloride are 20-40 μg./ml. and 2.5 μg./ml., respectively.[60] The effects of clamoxyquin hydrochloride and chloroquine against amebic hepatitis in hamsters were roughly comparable; clamoxyquin hydrochloride, pamoate, and salicylate were active against intestinal amebiasis in rats in gavage doses ranging from 75 to 600 mg./kg. daily for 4 days.[60] Clamoxyquin hydrochloride and pamoate cured amebic dysentery in dogs in daily doses of 3.13 to 50 mg./kg. and 12.5 to 25 mg./kg., respectively.[60] Relative to the 8-quinolinols in clinical use the clamoxyquin salts were deemed worthy of clinical



XIII



XIV

trial. Clamoxyquin hydrochloride was tolerated well in man following daily 15 mg./kg. doses for 5-10 days and was highly effective against various forms of intestinal amebiasis.[61] Additional studies will be required before the relative importance of the clamoxyquin salts in amebiasis chemotherapy can be assessed.

C. Anisomycin. - The determination of the structure and stereochemistry of the potent antiprotozoal antibiotic anisomycin (XIV)[62] may serve to stimulate future research on the synthesis and biological properties of related substances.

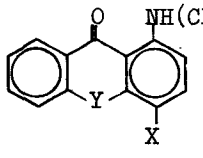
VI. SCHISTOSOMIASIS

Nearly 200 million people are infected with schistosomiasis, and on a world basis these infections are spreading.[4] The degree of prevalence, the seriousness of the infection, and the paucity of adequate drugs make schistosomiasis one of the greatest challenges in parasite chemotherapy today. Several promising new agents have been discovered, but their usefulness in man has yet to be adequately evaluated. Therefore, one still must rely on the antimonials with some assistance from lucanthone hydrochloride (XVa) against Schistosoma haematobium and certain strains of S. mansoni. Research on schistosomiasis[63,64] and other trematode infections[63] has recently been reviewed.

A. 2-Amino-5-nitrothiazoles. - To date 1-(5-nitro-2-thiazolyl)-2-imidazolidinone (Ambilhar®, 32,644-Ba) (XII) has been evaluated more extensively in schistosomiasis[55,57,65,67,68] than in amebiasis (vide supra). When adult S. mansoni were exposed to 32,644-Ba in vitro for prolonged periods (100 hr.), oviposition was inhibited at concentrations of 1 μg./ml. and worms were killed at 100 μg./ml.[55,65] The destruction of the vitellogenic gland in the female coincides with a reduction in body-length in both male and female worms and in the size of the ovary in the female.[57] The drug was highly active against S. mansoni[55,65] and S. japonicum[57] in mice in oral doses of 100 mg./kg. daily for 10 days and had a strong prophylactic and chemotherapeutic effect on experimental S. mansoni infections in monkeys.[57] Temporary, reversible impairment of spermatogenesis occurred in mice; the drug apparently acts directly on the germinal epithelium of the testes.[66]

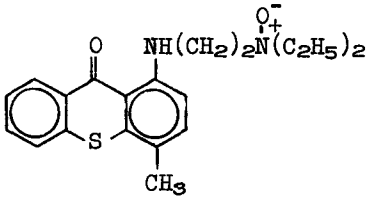
Results of preliminary clinical trials in man indicate that 32,644-Ba has considerable efficacy against S. haematobium[57,67] and S. mansoni[57,68] in oral doses of 20-40 mg./kg. daily for 5-15 days; S. japonicum infections appear to be more refractory.[57] Three types of side effects were encountered which merit special attention: (1) frequent ECG changes, involving mainly the T waves; (2) neuropsychic effects, ranging from severe headaches to convulsive attacks, with infrequent hallucinations; (3) transitory impairment of spermatogenesis.[57] The ultimate place of 32,644-Ba in schistosomiasis chemotherapy will depend largely on the incidence and severity of these side effects at effective dose levels when compared with the side effects encountered in the classical treatments. The drug has also been reported to be effective in the treatment of Dracunculus medinensis (guinea worm) infestation in humans.[69]

B. Thiaxanthenones. - Incubation of lucanthone (XVa) with Aspergillus sclerotiorum produces the acid-labile hydroxymethyl derivative (hycanthone) (XVb) together with smaller amounts of the aldehyde XVc.[70] Surprisingly, the five-day ED_{50} for lucanthone and hycanthone against S. mansoni in hamsters were 8.0 mg./kg. and 0.78 mg./kg., respectively.[70] The unusual potency of hycanthone led to the hypothesis that it might indeed be the elusive active metabolite of lucanthone. The acid sensitivity of XVb could explain why it had



	X	Y
a,	CH_3	S
b,	CH_2OH	S
c,	CHO	S
d,	CH_3	SO
e,	CH_3	SO_2

XV



XVI

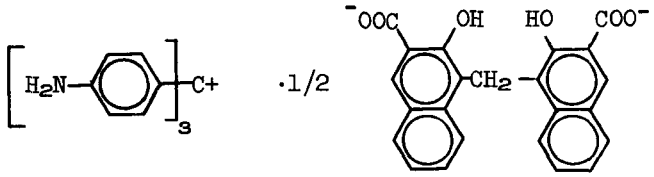
escaped detection by previous investigators. Accordingly, monkeys were medicated with lucanthone, the urines were carefully extracted avoiding acid treatment, and the extracts were examined by thin-layer chromatography. In agreement with earlier work,[71] the sulfoxide (XVd) was the major metabolite, and only traces of XVb were detected. When the urine extracts were incubated with glucuronidase prior to chromatography, however, hycanthone appeared as a major component.[70] Although the sulfone XVe is reported to be the major urinary metabolite in mice,[71,72] hycanthone was also found in mouse urine after glucuronidase incubation but to a much lesser extent than in the monkey.[70]

The results of these elegant studies support the hypothesis that hycanthone is an important active metabolite of lucanthone; it now becomes apparent why a p-toluidine function is an absolute requirement for activity among all lucanthone relatives. Other studies utilizing Bacillus subtilis suggest that the major in vivo action of lucanthone is to complex with DNA, thereby blocking DNA-dependent RNA synthesis.[73]

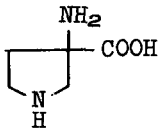
Various other schistosomicides related to hycanthone and the aldehyde XVc have been prepared.[74] It has also been disclosed that lucanthone N-oxide (XVI) is considerably less toxic than lucanthone.[75]

C. Tris(p-aminophenyl)carbonium Salts. - Tris(p-aminophenyl)carbonium (TAC) pamoate (XVII)[76] and certain antimonials have broad antischistosome activity in experimental animals[77] and in man.[78] However, both groups have important limitations. Thus the slow-acting TAC pamoate must be administered for several weeks to achieve a strong degree of effect while the fast-acting antimonials have a relatively narrow therapeutic index.

In a recent study of the joint action of TAC salts and tartar emetic a marked degree of synergism was observed against S. mansoni in vitro and in mice, particularly after pretreatment with TAC pamoate.[79] Moreover, neither drug had an appreciable affect on the gross toxicity of the other for normal mice. An encouraging degree of joint antischistosomal action by TAC pamoate and tartar



XVII



XVIII

emetic was also observed in monkeys. The therapeutic advantage of such a combination hinges largely upon whether or not the co-administration of TAC pamoate produces higher or more sustained antimony levels in the blood or tissues. A subsequent study utilizing ^{124}Sb-labeled tartar emetic demonstrated that co-administration or pretreatment with TAC pamoate had no significant effect on the disposition of antimony in mice or monkeys.[80]

Available evidence suggests that the mode of action of TAC pamoate and the trivalent antimonials is dissimilar. Briefly, the antimonials interfere with schistosome glycolysis by inhibiting phosphofructokinase, which catalyzes the reaction:

Fructose-6-phosphate + ATP ⟶ Fructose-1,6-diphosphate + ADP

Recent studies with ^{14}C-labeled glucose indicate that tartar emetic blocks both the Embden-Meyerhof and the hexose-monophosphate pathways of glycolysis.[81] Organic antimonials have also been reported to inhibit schistosome glutamic-pyruvic transaminase.

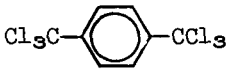
The TAC salts apparently act by an entirely different mechanism.[82] In mice subcurative doses of TAC chloride have no demonstrable effect on the carbohydrate and protein metabolism of the worms but produce a localized paralysis of the acetabulum, pharynx, and oral sucker which histochemically has been correlated with a pronounced decrease of cholinesterase activity in the central ganglia. This paralysis is completely reversed *in vitro* by the cholinergic blocking agent mecamylamine, suggesting an accumulation of acetylcholine in these structures. Simultaneously, glycogen depletion is observed in the papillae located below the cuticle of the worm. Either one or a combination of these changes could account for the antischistosomal effects observed.[82]

D. *Cucurbitine*. - For several years reports emanating from mainland China have claimed that the dried, ripe seeds of the pumpkin varieties *Cucurbita pepo* L. and *C. moschata* Duch. are effective in the treatment of schistosomiasis. The amino acid cucurbitine (XVIII) has recently been isolated from *C. moschata* Duch.[83] Cucurbitine reportedly inhibits the growth of immature *S. japonicum*, produces degenerative changes in the genital organs of the worms resulting in depressed egg-producing capacity, and is virtually nontoxic.[83] It would appear worthwhile to confirm the structure of cucurbitine and study its effects on *S. haematobium* and *S. mansoni*.

VII. CLONORCHIASIS

Clonorchiasis is caused by the human liver fluke *Clonorchis sinensis* (syn.

Opisthorchis sinensis) and is widely distributed in the Far East, especially in Indochina, Central and South China, Formosa, Korea, and Japan. Approximately 19 million people are afflicted with this disease, yet no reliable treatment is known. Therefore, preliminary reports that the fasciolicide α,α,α,α',α',α'-hexachloro-p-xylene (XIX) is highly effective against C. sinensis in experimen-



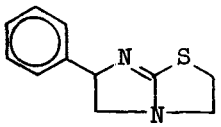
XIX

tal animals[84-86] and in man[86-90] at well-tolerated doses are most encouraging. Expanded human trials are indicated.

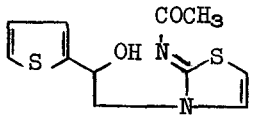
VIII. INTESTINAL HELMINTHIASES

The world-wide incidence of intestinal helminthic infections in man approaches the staggering figure of two billion.[4] 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.

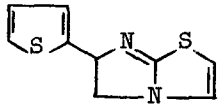
First reports[91] on tetramisole (XX), a potent new anthelmintic with broad action against nematodes, are highly encouraging. The drug is reported to be effective against Ascaris lumbricoides and Enterobius vermicularis in man[91] and against a variety of adult and immature gastrointestinal and pulmonary nematodes in laboratory animals, poultry, and livestock.[91,92] Although devoid



XX



XXI



XXII

of antihistaminic, anticholinergic, adrenolytic or other classical pharmacological properties, tetramisole exerts a rapid paralysing action on a variety of nematodes in vitro at low concentrations.[91]

The evolution of tetramisole points once again to the importance of strong biochemical support in drug development. Thus, the observation that thiazothienol (XXI)[93] was active against nematodes during a routine screening program in chickens stimulated a search for active metabolites of XXI and led to the isolation and identification of XXII as the major transformation product in the feces. The cyclic metabolite XXII was active at significantly lower dose levels that the parent compound (XXI), and these results prompted the synthesis and biological evaluation of an extensive series of related compounds.[93,94] Tetramisole emerged as the most promising substance for expanded studies.[91]

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Chapter 14b. Animal Antiparasitic Agents

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I. Introduction

Developments during 1965 in the field of animal health included reports of two new anthelmintics, tetramisole and bunamidine, which are representative of new chemical classes. Other reports described modifications of the older antiparasitic leads as well as new therapeutic applications of established agents. A number of investigations into the biochemistry of metazoan and protozoan parasites appeared, and the results of such studies may be hoped ultimately to point out new approaches in the search for new antiparasitic agents. Lack of space prohibits discussion of these papers in this survey, but pertinent references[1] are included for the use of interested readers.

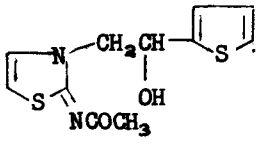
Two books which became available during 1965 are important additions to the literature of antiparasitic chemotherapy. Gibson's review[2] of veterinary anthelmintics covers the literature through 1962 and into 1963; and Schnitzer and Hawking's Experimental Chemotherapy, vol. IV[3], contains an appendix which supplements chapters in the earlier volumes dealing with trypanosomiasis, enterohepatitis, coccidiosis, babesiasis, theileriasis, anaplasmosis, filariasis, and myiasis.

II. Anthelmintics

A. Anthelmintics for Treatment of Roundworm Infections.

Tetramisole. Preliminary reports of an entirely new class of anthelmintics, hydrogenated derivatives of imidazo[2,1-b]thiazoles, appeared from the Janssen Laboratories[4,4a].

Discovery of the imidazo[2,1-b]thiazoles followed from the observation that 2-acetylimino-3-[2-hydroxy-2-(2-thienyl)-ethyl]-thiazoline (thiazothienol, I), under investigation as an anthelmintic in poultry, was metabolized to the more potent 6-(2-thienyl)-5,6-dihydroimidazo[2,1-b]thiazole (II)[4]. Further development of the lead led to 6-phenyl-2,3,5,6-tetrahydroimidazo [2,1-b]thiazole hydrochloride (III), for which the generic name tetramisole was proposed.



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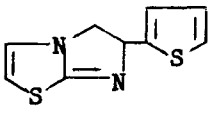

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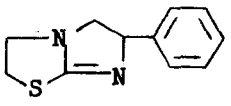


III
Tetramisole

Tetramisole was found by the Janssen group[4] to be a water-soluble (21% at 20°C.), broad-spectrum anthelmintic which can be administered orally, or by subcutaneous, intramuscular, or intraperitoneal injection. Doses of 40 mg/kg, orally or by injection, efficiently removed immature and mature forms of all three of the common chicken nematodes, Ascaridia galli, Heterakis gallinarum, and Capillaria obsignata. Toxicity to chickens was said to be low, doses up to 640 mg/kg being tolerated. Doses of 5-20 mg/kg, orally or by injection, were reported effective in the removal of a wide range of mature and immature gastrointestinal nematodes in sheep, as well as the lungworm, Dictyocaulus viviparus. Dose levels up to 40 mg/kg, orally, or 10 mg/kg., parenterally, were tolerated by sheep. Similar results were reported with cattle. The drug was active at 20 mg/kg, orally, against Toxascaris, Toxocara, and Uncinaria in dogs, but was poorly active against Trichuris. In all, these authors catalogued activity with tetramisole against at least 56 species of nematodes in thirteen hosts, including man. No activity against flukes (Fasciola hepatica) or tapeworms (Moniezia spp.) was observed.

Tetramisole was found to have a rapid paralyzing action in vitro against a variety of nematodes.

Walley[5] confirmed some of the observations described above concerning the efficacy of tetramisole. In tests with 1,076 sheep and 18 goats, tetramisole at 15 mg/kg gave good results against all mature and immature nematodes, with the exception of Trichuris. Adult worms were removed by a 5 mg/kg dose. Slight side-effects, consisting of hyperirritability and loss of appetite, were observed with the 15-20 mg/kg oral doses. These effects were more pronounced at doses above 50 mg/kg, and one sheep died after a 80 mg/kg dose. Clonic convulsions were induced by the highest levels.

These reports are preliminary and limited, hence final assessment of the efficacy and safety of tetramisole must await reports of wider investigations.

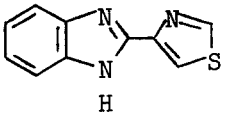
Thiabendazole. A detailed report concerning the toxicology and pharmacology of thiabendazole (IV) was contained in a paper by Robinson, Stoerk, and Graessle[6]. The oral acute LD_{50} of thiabendazole in mice, rats, and rabbits was 3600, 3100, and greater than 3800 mg/kg, respectively. In sub-acute toxicity tests, mice and rats grew normally when fed thiabendazole in the diet for 30 days. At levels of 0.2%, 0.4%, or 0.6% in the ration, or with single oral doses of 100 or 400 mg/kg for 30 days, all mice and rats survived; and, except for reductions in food consumption and weight gain, no outward signs of toxicity were seen. The effective oral dose of thiabendazole against nematode infections in man and animals has been found to be in the range of 25-50 mg/kg.[6,7]

Thiabendazole was reported to be effective in the eradication of Hyostrongylus rubidus in pigs, using a single oral dose of 100 mg/kg.[8]

Thiabendazole was active against the larval stages of Capillaria obsignata infections in chickens at 1000 mg/kg, but was not effective against mature parasites at this high dose.[9]

Trials were conducted with thiabendazole in the treatment of nematode infections in horses.[10,11] At 44 mg/kg and 88 mg/kg, orally, the drug was effective against immature and mature small strongyles and against Probstmayria vivipara and Oxyuris equi.

Campbell and Timinski[12] observed development of immunity to Ascaris in rats cured of this infection by treatment with thiabendazole.



IV Thiabendazole

Other Benzimidazoles. Deaths of sheep following the use of 2-phenylbenzimidazole at 250 mg/kg under conditions of reduced water intake was investigated by Jones, Leaver, and Milne.[13] Toxicity was attributed to kidney damage caused by deposition of a crystalline metabolite, most likely 5-O-glucuronyloxy-2-phenylbenzimidazole. The pH of the urine and rate of urine output were thought to be critical factors affecting deposition of the metabolite. A pH of 8.4 or higher was considered important to protect animals against the deposition phenomenon when water intake was low. Use of 2-phenylbenzimidazole formulations containing alkalizing ingredients, such as potassium citrate[14], sodium carbonate[14], or magnesium salts[15] was proposed by the manufacturer (Imperial Chemical Industries) to minimize the risk of toxicity with this anthelmintic.

Preliminary reports of other new anthelmintic benzimidazoles have appeared, although details of biological testing are not yet available[16-19].

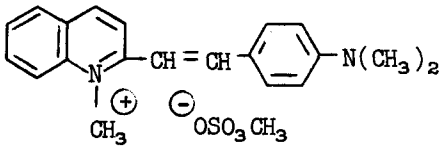
Quaternary Ammonium Anthelmintics. A group of related quaternized heterocyclic compounds have been under investigation for the treatment of Ascaris infections in pigs and hookworms in dogs.

In earlier reports[20,21], it was found that 1-methyl-2-(p-dimethylaminostyryl)-quinolinium methosulfate (V) protected pigs from liver damage caused by experimental infections with migrating ascarids. At a level of 0.014% in the diet, the drug lessened the severity of the infection. A level of 0.056% completely prevented liver lesions when administration of the drug was started prior to establishment of the infection. This lead has now been extended to the styrylpyridinium analog[22] (VI), and to certain phenylbenzothiazolium salts (VII).[23] Diet levels of approximately 0.02%, or lower, of VI or VII prevented liver damage in Ascaris-infected pigs, when medication was initiated two days before inoculation with Ascaris larvae.

In a parallel development, 2-(p-chlorostyryl)-pyridinium salts (VIII) were reported to be highly effective against mature and immature hookworms (Ancylostoma caninum) in dogs at 5 mg/kg, and could be given in the ration, as well as by capsule[24,25].

The benzdithiylium salts IX and X, described by Wizinger and Soder[26], and for which broad anthelmintic activity was claimed, would seem to conform to this same general lead.

Insufficient data have been published to allow final conclusions about the safety and utility of these compounds.



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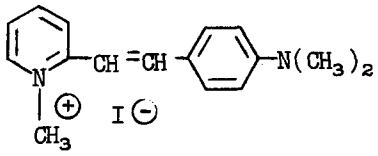
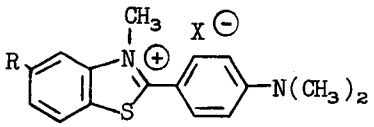
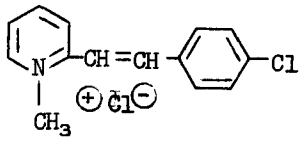


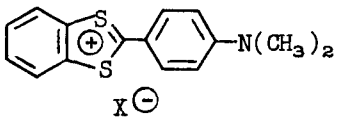
VI



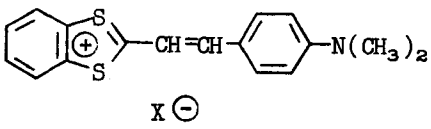
VII, R = H or CH_3



VIII



IX



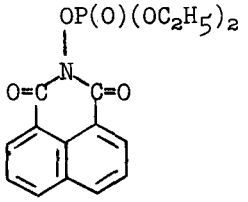
X

Organophosphate Anthelmintics. Several additional studies of the use of the organophosphates phthalophos[27,28] (0,0-diethyl-0-naphthaloximide phosphate, XI) and haloxon[29,30] (0,0-di-(2-chloroethyl)-0-(3-chloro-4-methyl-7-coumarinyl)phosphate, XII) appeared during the year.

Phthalophos was found by Hebden and Hall[27] to be completely effective against Haemonchus contortus, Ostertagia spp., Trichostrongylus axei, and T. colubriformis in sheep at 50 mg/kg (natural infections). At this level, phthalophos had variable (38-94% removal) efficacy against Nematodirus species, and poor activity against the large bowel worms Oesophagostomum columbianum and O. venulosum. In parallel tests, thiabendazole was completely effective in the removal of Oesophagostomum species at 50 mg/kg.

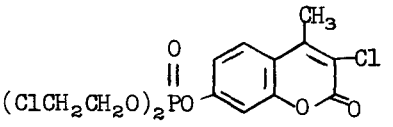
Studies in cattle[28] showed phthalophos to be highly effective against Haemonchus, Ostertagia, Trichostrongylus, and Cooperia species at 50 mg/kg and 75 mg/kg, and to reduce the levels of Bunostomum infections at these doses. Hebden and Hall[27] found no toxic effects with phthalophos in sheep at up to six times the effective doses (i.e., at up to 300 mg/kg), and reported only a transient diarrhea at 400 mg/kg.

In trials with haloxon, Baker[29] confirmed earlier reports of the anthelmintic effectiveness and relative safety of this substance in sheep at 50 mg/kg. Efficiency against Ostertagia circumcincta and Trichostrongylus vitrinus were 95% and 98%, respectively, though Nematodirus spathiger was not removed by this drug at 50 mg/kg.



XI

Phthalophos



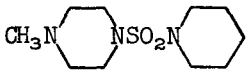
XII

Haloxon

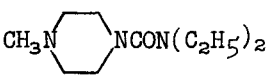
Other Nematocides. A new piperazine derivative[31,32] (1-methyl-piperazine-4-sulphonylpiperidide, XIII), a sulphonyl analog of diethylcarbamazine (XIV), was found by Teuscher[31] to eliminate lungworm (Dictyocaulus filaria) infections in sheep. The drug was administered in the form of two consecutive daily 20 mg/kg doses, subcutaneously. The toxicity of XIII relative to diethylcarbamazine was not reported.

Of a series of standard anthelmintics studied for the control of experimental Capillaria obsignata infections in chickens, methyridine (XV) proved highly effective against both mature and immature parasites at doses of 100-150 mg/kg, given subcutaneously. Complete eradication of Capillaria

infections was achieved with 200 mg/kg of methyridine by the oral route[33]. Haloxon was found fully effective against adult Capillaria at 50-60 mg/kg in this study, whereas efficiency against immature parasites was not satisfactory.

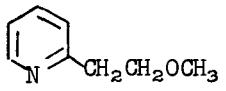


XIII



XIV

Diethylcarbamazine



XV

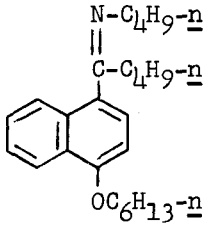
Methyridine

B. Anthelmintics for Treatment of Tapeworm Infections.

Bunamidine. Bunamidine (N,N-dibutyl-4-hexyloxynaphthamidine, XVI) is representative of a new class of cestocidal agents[34,35]. In initial experiments, the drug was reported very effective against Taenia pisiformis, orally, at 25 mg/kg, in the dog[35]. The same substance partially cleared Dipylidium caninum in dogs, and some variable effect against the ascarid Toxascaris leonina was reported. In cats, bunamidine at a dose of 25 mg/kg resulted in the complete elimination of Taenia taeniaformis. Cats with concurrent ascaris infections (Toxocara cati) experienced an average of 80% clearance of this parasite with this dose of bunamidine.

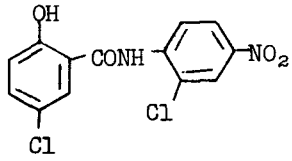
The preliminary announcement disclosed activity against other species of tapeworms in cats, dogs, and sheep, but without details of the tests. Good activity against Echinococcus granulosus in dogs was reported as an especially important quality of bunamidine.[34]

Side reactions in dogs and cats consisted of occasional emesis and diarrhea with 25 or 50 mg/kg doses. Chronic treatment of pregnant dogs with 100 mg/kg /day of bunamidine starting four weeks prior to delivery was not harmful to the mothers or pups.[35]



XVI

Bunamidine



XVII

Niclosamide

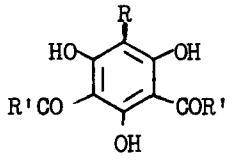
Niclosamide. Strufe[36] reported data on the effect of niclosamide (5,2'-dichloro-4'-nitrosalicylanilide, XVII) on the metabolism of Hymenolepis diminuta. The compound was found to interfere with glucose utilization in tissue homogenates prepared from this parasite.

In a critical trial with niclosamide in dogs infected with Taenia species, clearance was obtained with 100-200 mg/kg.[37]

Male Fern Constituents. Blakemore, Bowden, Broadbent, and Drysdale[38] isolated some of the phloroglucinol derivatives from Dryopteris rhizomes and tested them individually for cestocidal activity. Flavaspidic acid, aspidin, and desaspidin were active orally against Hymenolepis nana in mice, but the monocyclic phenol aspidinol was not active at this level.

In later papers[39,40], the SKF group synthesized simpler monocyclic phloroglucinol derivatives related to Dryopteris constituents. Compounds of the type XVIII were active against experimental H. nana infections in mice orally at 400 mg/kg. Tests with lower doses were not reported. Many other members of the series showed a lower order of anthelmintic activity.

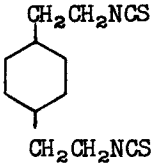
Cyclohexane-1,4-bis-(2-ethylisothiocyanate) (XIX). This compound was studied in 319 sheep infected with tapeworms[41]. A 25 mg/kg dose was considered adequate in the treatment of simple Moniezia expansa infections. For multiple cestode infections, a level of 100 mg/kg dose was recommended. Activity against other species of tapeworms was reported as well. The compound was said to be well-tolerated, the minimum lethal dose in sheep being about six times the highest recommended dose of 100 mg/kg.



XVIII

R = H or CH_3

R' = i-C_3H_7; n-C_4H_9; n-C_5H_{11}; n-C_6H_{13}; $-CH_2CH_2CH(CH_3)_2$.



XIX

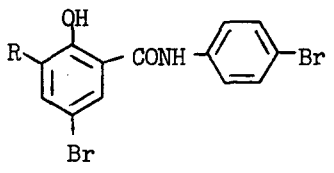
C. Anthelmintics for Use Against Liver Flukes.

Salicylanilides. The use of Diaphene®, a mixture of three parts 3,5,4'-tribromosalicylanilide (XX) and one part 5,4'-dibromosalicylanilide (XXI), for the treatment of liver fluke disease in cattle was first proposed by Lienert[42,43].

Hilomid® (Astra Pharmaceuticals), a 1:1 mixture of XX and XXI, was reported by Boray, Happich, and Andrews[44] to be effective (86-98%) against 10-12 week old Fasciola hepatica infections in sheep when administered in oral doses of 30 mg/kg. The higher doses of 60 mg/kg resulted in 90-100% removal of larger immature flukes (six weeks or older). These authors observed that mortality due to acute liver fluke disease usually occurs after seven or eight weeks from the time of infections, and hence Hilomid® may prove effective in controlling F. hepatica infections.

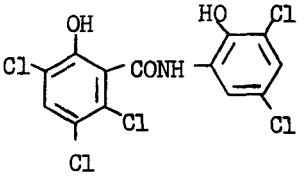
Salicylanilides related to Diaphene® and Hilomid® were described in a patent by Stecker[45].

A series of chlorinated 2'-hydroxysalicylanilides was described in ICI patents[46,47]. One member of this series, 3,3',5,5',6-pentachloro-2,2'-dihydroxybenzanilide (oxyclozanide, XXII) was studied in 1226 sheep and 530 cattle by Walley[48]. A 15 mg/kg oral dose was recommended for treatment of sheep, and 10-15 mg/kg, orally, for cattle. The author observed a four-fold margin of safety in normal animals, but a narrower margin in dehydrated animals, or those suffering from liver damage. The stated doses were reported effective for the eradication of mature flukes. Substantially higher doses (50-60 mg/kg) removed a high proportion of immature (over six weeks) flukes, but these doses are in the toxic range. Toxic side-effects were observed in doses above 30 mg/kg, with deaths occurring at 60 mg/kg. (sheep and cattle).



XX, R = Br

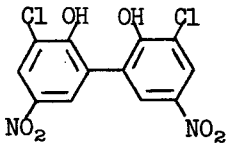
XXI, R = H



XXII

Oxyclozanide

Other Fasciolacides. Bayer 9015 (2,2'-dihydroxy-3,3'-dichloro-5,5'-dinitrobiphenyl, XXIII)[50,51] appears to be the most potent member of the group of anthelmintic bisphenols which includes hexachlorophene, bithionol, and oxyclozanide. In two recent reports, it was found effective against mature liver flukes at 5 mg/kg[50], and at 6 mg/kg [51] in sheep. In other critical trials[50], 93-100% clearance of mature parasites was achieved with 3 mg/kg, orally, in 21 sheep. No gross toxicity due to the drug was reported at these dose levels, although some reduction in rate of weight gain was seen in some experiments at 6 mg/kg. Lee, O'Nuallain, and Power[51] indicated that the manufacturer had observed non-fatal toxic reactions with Bayer 9015 in 20 sheep dosed with 20 mg/kg. Bayer 9015 at 5 mg/kg exhibited 40-57% efficiency against immature flukes up to 10 mm in length, and 53-100% activity against larger immature forms[51]. The drug was ineffective against the lancet fluke Dicrocoelium dendriticum in one sheep which harbored this parasite.



XXIII

A series of novel organophosphates was reported in Bayer patents to possess activity against liver flukes in rats and sheep at 5-15 mg/kg, orally and intramuscularly[52,53].

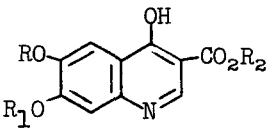
Kondos and McClymont[54] produced evidence that the fasciolacidal activity of carbon tetrachloride is indirect. Anthelmintic effect of the drug in vitro was low but was enhanced greatly by addition of fresh liver slices or a minced liver preparation. The in vivo activity of CCl_4 was much higher than could be explained by the observed in vitro effect of the substance. The anthelmintic activity was attributed to an unknown metabolic product or products.

Although the range of anthelmintics active against the liver fluke is widening, the goal of an agent active against immature flukes at safe dose levels appears yet to be achieved. Some of the phenolic anthelmintics described in the papers cited above represent progress in this direction, in that some effect against larger immatures was seen at elevated dose levels, but the reported toxicity of the products would limit or prohibit use of these higher doses.

III. Antiprotozoal Agents

A. Coccidiostats

Quinolines. Reference to anticoccidial quinolines appeared in a number of patents by Norwich. Compounds of the type XXIV were said to be effective in the control of Eimeria tenella, E. acervulina, and E. necatrix infections at diet levels between 0.006% and 0.1%[55,56]. Details of biological testing have not yet been published. Buquinolate (XXIV, R=R_1=i-butyl, R_2= ethyl) has been proposed for treatment of coccidiosis in broiler chickens at a concentration in feed of 75 grams per ton (0.00825%).[57]

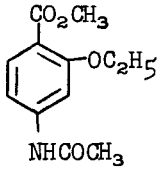


XXIV

R and R_1 are ethyl, propyl, or butyl.

R_2 is lower alkyl.

p-Aminobenzoic Acid Antagonists. Exceptional anticoccidial activity was found with methyl 4-acetylamino-2-ethoxybenzoate (ethopabate, XXV).[58] This substance was reported active against E. maxima in chickens at 0.0001% in the diet. Coccidiostatic activity of ethopabate was reversed by simultaneous administration of p-aminobenzoic acid. Ethopabate was also found active against E. brunetti and E. acervulina, but a level of 0.01% in the feed was ineffective against E. tenella.



XXV

Ethopabate

Ryley[59] examined the efficacy of a combination of 1,1'-dimethyl-4,4'-bipyridylium dichloride and sulfamethazine for the control of outbreaks of coccidiosis in chickens. The combination, known by the ICI trademark Paramez®, was used in a final concentration in water amounting to 0.02% sodium sulfamethazine and 0.0625% of the bipyridylium dichloride. Paramez® was said to be effective against Eimeria brunetti, E. maxima, and E. acervulina at this concentration without diminishing rate of growth of the birds. The bipyridylium compound alone was not effective at non-toxic levels. The author speculated that the lower level of sulfa in this combination should reduce the risk of development of the haemorrhagic syndrome which has been observed with the use of sulfonamide coccidiostats.

Ball and Warren[60] reviewed the history of sulfonamide combinations as coccidiostats and studied combinations of 4,4'-diaminodiphenylsulfone (DDS) with 2,4-diamino-5-(4-chlorophenyl)-6-ethylpyrimidine (pyrimethamine) and with 2-amino-4-dimethylamino-5-(4-chlorophenyl)-6-ethylpyrimidine hydrochloride (May and Baker 4408), and also combinations of pyrimethamine with sulfaquinoxaline. Potentiation was seen with all of these combinations, but the final utility was not evaluated.

Other Coccidiostats. Preliminary reports in patents claimed anticoccidial activity for sulfonylurethanes[61,62], lincomycin[63], γ-pyridones[64], benzoquinones[65], and nitropyrroles[66].

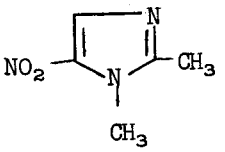
Nicotinamide antagonists (6-aminonicotinamide, pyridine-3-sulfonamide, and 3-acetylpyridine) were reported by Ball, Warren, and Parnell[67] to possess coccidiostatic activity. Similar observations have been made by McManus, Clark, Rogers, and Cuckler[68], who noted toxicity of these compounds in chickens.

B. Histomonostats

A study by Flowers, Hall, and Grumbles[69] confirmed earlier reports of the utility of 1,2-dimethyl-5-nitroimidazole (dimetridazole, XXVI) for the

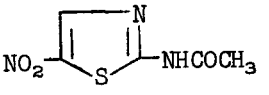
prevention and treatment of enterohepatitis in turkeys. The compound was judged equal to or better than 2-acetylamino-5-nitrothiazole (Enheptin-A) for the prevention of enterohepatitis.

5-Nitro-2-furaldehyde acetylhydrazone (XXVII) was found by Hall, Flowers, and Grumbles[70] to be efficacious (comparable to Enheptin-A and dimetridazole) in the prevention of losses from enterohepatitis when administered prophylactically in the feed at 0.022%.

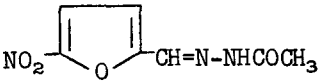


XXVI

Dimetridazole



Enheptin-A

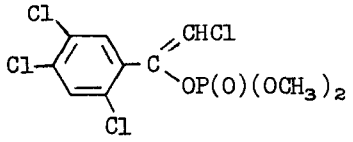


XXVII

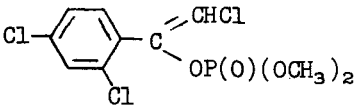
Lucas and Goose[71] presented a detailed description of a screening procedure for antiblackhead agents, using chickens infected with Histomonas meleagridis. Use of chickens instead of turkeys for screening was said to be more convenient and economical.

IV. Organophosphate Insecticides

A promising new organophosphate insecticide was announced by the Shell group[72]. This compound, an α-phenylvinyl phosphate (XXVIII) was said to possess good activity against several species of insects, and its acute oral LD_{50} was greater than 4000 mg/kg in mice and rats. It is close in structure to chlorfenvinphos, (XXIX), which was evaluated in field trials for the protection of sheep against the maggot fly, Lucilia sericata[73]. Chlorfenvinphos was judged equivalent in efficacy to dieldrin at comparable concentration in a dip (0.05% compared with 0.04% dieldrin).



XXVIII



XXIX

Details of the synthesis and testing (mouse-mosquito assay and mouse toxicity tests) of a series of phosphorylated benzenesulfonamides in the famophos series were presented by Wagner, Baer, and Berkelhammer[74].

Trials with various older organophosphates and other insecticides were reported by Sharma, Chhabra, and Kapoor[75] (cattle grubs), Unwin[76] (cattle grubs), Drummond and Medley[77] (cattle ticks), and by Drummond and Graham[78] (cattle grubs, Oestrus ovis in sheep, and Gastrophilus species).

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Section IV - Metabolic Diseases and Endocrine Function
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Chapter 15. Antidiabetic Agents

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The hypoglycemic drugs that are used today to treat well over a million diabetics are descended in a direct line from two essentially chance observations -- both made more than 20 years ago. The transition from these observations (that certain antibacterial sulfonamides caused hypoglycemia[1] and that a simple guanidine derivative reduced the blood sugar of rabbits[2]) to safe, effective therapy is a real achievement of medicinal chemistry. Nevertheless, it would be unrealistic not to recognize that in spite of the vast body of fact and conjecture now contained in the literature of diabetes, metabolic biochemistry and the control of metabolism; no antidiabetic drug has yet come from rational application of knowledge about these processes. Compounds unrelated to the sulfonylureas or guanidine derivatives have, to be sure, been studied for their hypoglycemic properties -- some extensively -- but all were apparently first identified by a largely empirical observation of their effect on blood sugar in experimental animals.

Brief sections on diabetes as a disease entity and on some of the hormonal factors and metabolic processes that are deranged in diabetes have been included. Limitations both of space and of the talents of the reviewer preclude either comprehensive or profound treatment of these aspects and nothing approaching this has been attempted. Rather, the aim is to invite attention again to the relationship of these factors to the disease and to provide a few leading references that may stimulate thought on possible new approaches to antidiabetic drugs.

Diabetes Mellitus

Diabetes is a hereditary disease[3,4] but the nature of the genetic defect and its primary lesion are unknown. It involves a widespread disorder of metabolism but exhaustive study has not yet determined whether this is a cause or consequence of the hormonal, vascular and neurological abnormalities of the disease. Reasonably up-to-date treatment of all of these aspects appear in the symposium volume from the Fifth Congress of the International Diabetes Federation (Toronto, 1964).[5] A chapter on the unanswered questions about human diabetes by Williams and Wood[6] and the discussion that followed its presentation contain a host of suggestions for reading. Another review by Williams,[7] and Berson's 1965 Banting Memorial Lecture[8] are also helpful guides to current thought on diabetes.

Insulin

The dominant characteristic of diabetes is an absolute or relative lack of effective insulin action. In some manifestations of the disease, a deficiency of pancreatic β-cell function is clearly involved, but in others, the defect appears most likely to be a factor or condition that prevents insulin action.

Cahill has written an outstandingly readable account of insulin effects and their deficiency in diabetes.[9] A review of the insulin literature to July 1965[10] covers many aspects of insulin structure, synthesis, storage and release but does not cover insulin action. The review of structure, partial and total synthesis, and the influence of structure on activity is quite exhaustive. Events that occur during insulin release from the pancreas have been observed by electron microscopy,[11] and much is also known about β-cell metabolism.[12] The details of the process by which glucose actually triggers insulin release, however, are not yet understood in any detail.

One of the most fundamental activities of insulin is the removal of a barrier to the entry of glucose into fat and muscle cells. The functions of insulin in glucose[13,14] and amino acid[15] transport and the role of sodium and potassium ions in these processes[16] are the subject of a recent symposium. Important though the transport effect of insulin is, it is clear that it is not the primary action from which all other effects result. Some actions of insulin, as of most hormones, have been traced to effects on the synthesis of enzyme protein. Some of these effects have been postulated to occur at the level of DNA directed RNA synthesis, whereas others affect processes not yet clearly identified. Insulin effects on liver glycogen synthesis and breakdown[17] have been explained as part of a general theory of insulin induction of glycolytic enzymes and suppression of gluconeogenic enzymes,[18] Regulation of glucokinase and hexokinase activity depends on glucose concentration for immediate control, but on insulin induced de novo enzyme synthesis, apparently through control of new RNA production, over the longer term.[19] A similar effect appears to be responsible for insulin induction of saturated and monounsaturated fatty acid synthesizing enzymes.[20,21] Evidence has also been obtained that insulin stimulation of hepatic protein synthesis is due to an as yet undefined effect on ribosomes.[22]

Plasma factors that influence insulin action. Patients treated with insulin develop insulin antibodies, and there has been much speculation, now largely discounted,[8] that an autoimmune reaction may be involved in the etiology of diabetes. Immune insulin resistance, as a therapeutic problem, occurs in only a small minority of patients.[23,25] Considerable research has been devoted to structural modifications of insulin in an effort to reduce antigenicity.[10] Even homologous insulins, however, have proved to be antigenic, if administered in particular ways.[24]

It is now beyond dispute that there exist in plasma, substances other than pancreatic insulin and insulin antibodies that either have insulin-like activity or are capable of modifying the action of insulin.[25] The chemical identity and especially the physiological significance of these plasma constituents, however, still remains obscure. The substantial amount of research on these substances over the last five or six years stems from two observations; 1) that the amount of insulin-like activity assayable in plasma by using adipose tissue as the assay system is much greater than when muscle is employed and that both show more insulin-like activity than can be detected by radioimmunoassay[31,32] and 2) that various albumin fractions isolated from plasma are capable of inhibiting the effect of insulin on isolated muscle.

The first observation has been pursued by several groups, most extensively by Antoniades,[26,27] Samaan and Fraser,[28,29] and Froesch.[30] Antoniades and his co-workers have based their investigation on the fractionation of plasma, using an ion exchange resin, into "free" and "bound" insulin. The "free" fraction is apparently pancreatic insulin, since it is detectable by radioimmunoassay and stimulates glucose uptake by muscle. The "bound" fraction is not detectable by radioimmunoassay, stimulates glucose uptake by adipose tissue but not by muscle (unless an adipose tissue extract is added). Samaan and Fraser also distinguish two types of insulin-like activity. One, which they term "typical", is inactivated by insulin antibodies whereas the other, or "atypical" insulin is not. Froesch and his collaborators, working along lines very similar to Samaan and Fraser, classify the two kinds of plasma insulin-like activity as antibody "suppressible" and "non-suppressible." Davidson has studied a material with insulin-like activity on the rat diaphragm that is obtained from plasma by acid ethanol treatment.[33] Power is working with a serum protein which has insulin-like activity and augments the action of insulin on the rat fat pad and which may prove to have relevance to the theories of Antoniades.[34]

The chief difference between "atypical" or "non-suppressible" insulin, on the one hand, and Antoniades "bound" insulin on the other, is that the plasma concentrations of the former do not vary in response to changing plasma glucose levels, but the latter does. From his work, Antoniades concludes that the plasma insulin system he describes has a physiological role in the modulation of insulin activity. He visualizes a system in which insulin becomes bound or unbound in response to physiological stimuli and that diabetes may represent, in part at least, a disorder of this system. Although Antoniades observations have been confirmed,[35] his interpretations have been challenged, especially by Berson.[8]

Froesch appears to be making the most progress toward characterizing "bound" or "non-suppressible" insulin. He and his colleagues are working toward the isolation and characterization of this fraction. Their data thus far suggest that it is a small peptide, not identical with pancreatic insulin, with a molecular weight of about 6000, which is complexed with a large basic protein with a molecular weight over 100,000.[30]

From present evidence, it is difficult to ascribe a physiological function to these substances. It seems clear that they are not pancreatic insulin in equilibrium with a carrier protein complex as are many hormones such as cortisol and thyroxine. Plasma concentrations (except for Antoniades "bound" insulin) do not vary and the activity is not found in lymph, where immunoassayable insulin levels closely parallel those in plasma.[124] Perhaps the isolation work Froesch is now doing will clarify their status.

Vallance-Owen first reported that dilute solutions of an albumin fraction were capable of inhibiting the effect of insulin on the rat diaphragm and further showed that albumin isolated from the plasma of diabetics was capable of inhibiting insulin action at a lower concentration than was the corresponding fraction from non-diabetics.[36] These observations have now been confirmed,[37,38] but Vallance-Owen's hypothesis that the inhibitor, which he named synalbumin, is the albumin bound B-chain of insulin now seems highly

questionable. Mixtures of B-chain and albumin inhibit glucose uptake in diaphragm only at concentrations of B-chain many fold higher than seem possibly attainable in plasma.[39,40] Because the albumin insulin inhibitor is found in apparently higher concentrations in the plasma of diabetics than in the plasma of non-diabetics, Vallance-Owen has suggested that this substance may be involved in the etiology of diabetes.

The physiological significance of the Vallance-Owen factor seems highly dubious. Undiluted plasma has no inhibitory effect on diaphrams *in vitro* and no effect has been detected *in vivo*. Moreover, intravenous infusions of insulin B-chain have no effect on blood sugar.[8]

Other Hormones and Humoral Factors

Several other hormones have been studied intensively in connection with diabetes because of their effects on metabolism and insulin action.[6] Growth hormone and the glucocorticoids have often been classified as "diabetogenic" because their effects generally oppose those of insulin. Growth hormone depresses the phosphorylating capacity and insulin responsiveness of skeletal muscle and stimulates glucose oxidation in adipose tissue without altering its responsiveness to insulin, but the mechanism of these effects has not been established. The evidence obtained thus far, however, is consistent with the view that they are the result of the lipolysis stimulating effect of growth hormone.[41]

The glucocorticoids markedly increase the activity of key enzymes of gluconeogenesis and some evidence suggests that this effect is due, in part at least, to increased enzyme system synthesis resulting from a stimulation of the production of messenger RNA.[42] Other evidence, however, does not entirely support this hypothesis.[43]

Glucagon and epinephrine both oppose the action of insulin by stimulating glycogenolysis and gluconeogenesis through a cyclic 3',5'-AMP mediated process.[44] In addition, the hyperglycemic action of epinephrine is augmented by two other actions; a direct inhibition of insulin release from the pancreas[45,46] and a stimulation of lipolysis in adipose tissue, thus increasing the circulating level of free fatty acids, which oppose insulin action.[47,48] Glucagon, although it has the same effect as epinephrine on glycogenolysis, has now been shown to have the opposite effect on the β-cell, where it stimulates the release of insulin directly.[10] A symposium on the metabolic effects of catecholamines from the Second Catecholamine Symposium (Milan 1965) has appeared.[49]

Crude preparations of an exercise factor[50] that can apparently increase glucose utilization in muscle have now been obtained by Goldstein. From Sephadex column behavior, the material appears to have a molecular weight of less than 1000.[51]

Intermediary Metabolism

A wide ranging disorder of energy metabolism is characteristic of diabetes. Whether this derangement is caused by insulin deficiency or wholly

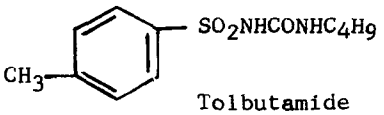
or in part by some other lesion remains to be learned, as does whether it is a cause or a consequence of the vascular complications of the disease. Nevertheless, it remains the facet of the disease to which therapy has been directed and by which the therapy is guided, whether it be diet, insulin, sulfonylureas or biguanides.

The hormonal and feedback control of metabolism has been extensively discussed by several authors, but particularly clearly by Randle.[52] He points out that there is a simple, primitive feedback control of metabolism, exerted through the effect of metabolic intermediates and substrates on enzymatic processes and that this mechanism of control is important, not only to simple organisms that depend on it entirely, but also to complex organisms which also have an additional component of hormonal control. That effective management of diabetes can be obtained by drug effects on either hormonal or feedback control of metabolism is attested by the clinical success of the sulfonylureas and biguanides.

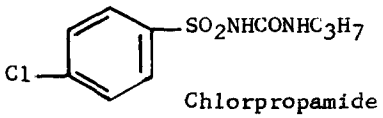
The role of all members of the trinity of energy metabolism; the liver, muscle and adipose tissue, has been extensively studied. Most of the current knowledge about adipose tissue metabolism is contained in a recent volume in the Handbook of Physiology series[53] and has also been discussed by Renold in a recent Banting Memorial Lecture.[54] The elegant and very useful quantitative work from Ball's laboratory on the fate of glucose carbon in adipose tissue has been summarized.[55] These researchers have recently shown that, in adipose tissue, the operation of citrate cleavage enzyme and malic enzyme together can provide both the extramitochondrial acetyl CoA and the $NADPH_2$ required (in addition to that from the pentose shunt) for lipogenesis.[56] The control of metabolism in muscle has been reviewed by Randle.[52]

Sulfonylureas and their Congeners

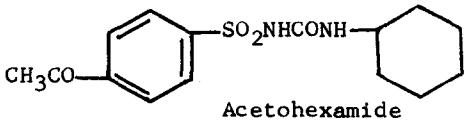
In the United States, three sulfonylureas are in wide clinical use in the management of maturity onset diabetes; tolbutamide (Orinase), chlorpropamide (Diabinese) and acetohexamide (Dymelor). In other parts of the world, carbutamide (Nadisan) and glycodiazine (Redul), a sulfonamidopyrimidine isostere of the sulfonylureas are also used.



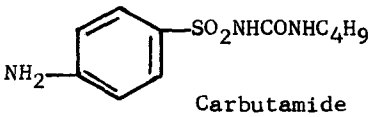
Tolbutamide



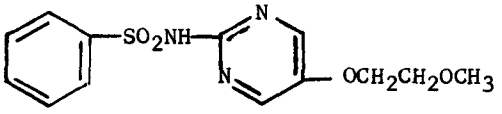
Chlorpropamide



Acetohexamide

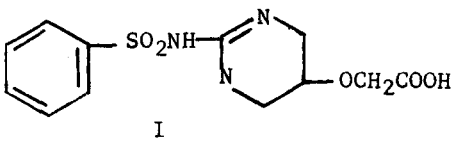


Carbutamide

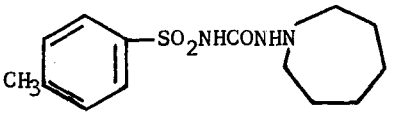


Glycodiazine

Glycodiazine[57] is one member of a large group of closely related compounds. In its pharmacology and clinical application, it appears to resemble tolbutamide most closely, having approximately the same hypoglycemic potency and duration of action.[58] The hypoglycemic effect of glycodiazine is probably due to the action of the compound itself, since the pyrimidine ring has proved to be metabolically stable. The principal metabolite is the carboxylic acid (I) that results from cleavage of the terminal side chain methoxyl group and oxidation of the resulting primary alcohol. A toxicology investigation has also been reported.[57]



I

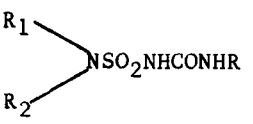


Tolazamide

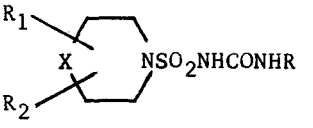
New sulfonylureas and their congeners continue to be widely reported, but in many cases, apparently from work done some time ago. It is regretable that there is no current structure-activity review, but one is clearly beyond the scope of this discussion. A compilation of a number of compounds that have been studied in animals and humans appeared a few years ago and is still useful.[59]

Sulfonylsemicarbazides have also been found to have significant hypoglycemic activity and one, Tolazamide,[60,61] has received FDA approval,[62] but had not, at this writing, been marketed.

An extensive series of hypoglycemic sulfamylureas (II, III) has been described.[63] In general, maximum hypoglycemic activity was found in



II



III

X = CH_2, N, O, S

R = alkyl, cycloalkyl and haloalkyl

R_1 and R_2 = alkyl and alkoxy

this series among the same alkylurea substituents (R) that give maximum activity in the sulfonylureas. The corresponding sulfamyl semicarbazides[64] also had significant hypoglycemic activity as did a few of the related sulfamylcarbamates.[65] Compounds in which the alkylurea portion was derived from a secondary amine, usually heterocyclic, were, in general, less active.[65] A number of the compounds were as active (reduction of blood glucose in fasted rats) as chlorpropamide. Further details of the pharmacology of the sulfamylureas have not been reported, but it seems safe to assume that their mechanism of hypoglycemic action would be very similar to that of the sulfonylureas. Several of the compounds are stated to show hypoglycemic activity in man but their clinical use in the management of diabetes has not been reported.

This work made good use of the now firmly established correlation, among compounds of this class, of hypoglycemic action with the presence of the compounds in the blood. Synthesis was therefore guided not only by the relationship established between structure and acute hypoglycemic activity, but also by an evolving concept of the influence of physical and chemical properties on absorption, metabolism and excretion, derived from a concurrent investigation.[66] The metabolism and excretion of 15 of the compounds were determined in dogs and seven were studied in human subjects. The results of these studies were correlated with physical and chemical properties, of which pK_a and lipid-water partition ratio proved to be the most useful.

Other variants on the sulfonylurea structure have recently included p-alkenyl-,[67,68] p-acyl-,[69] p-alkoxyalkyl-,[69] p-haloalkyl-,[69] and p-cyanobenzenesulfonyl-[69] as well as 6-sulfamoylbenzotriazole,[67] 6-sulfamoylbenzimidazole[67] and 5-indanylsulfonyl[70] derivatives. A variety of alkyl- and aralkylurea[71] substituents have been combined with these as well as with the now conventional benzenesulfonyl moieties. A series of compounds in which the urea function was part of a benzimidazolinone ring were devoid of hypoglycemic activity.[72]

A sulfonamidothiadiazole very similar to the sulfonamide[1] that started it all has been reported to reduce blood sugar in alloxan diabetic rats, but the experiment was not controlled with a known sulfonylurea.[73] Considering its structural similarity to the earlier compound, incomplete alloxanization appears a likely explanation of this result.

Pharmacology, Mechanism of Action. The pharmacology of the sulfonylureas has been reviewed recently.[74,10]

It is now almost universally accepted that the primary action of the sulfonylureas is to release insulin from the pancreatic β-cells. Overwhelming evidence indicates that the drugs have no significant hypoglycemic effect in animals or human diabetics who are without functioning β-cells. The details of the process by which the sulfonylureas release insulin, however, remain as much a mystery as the process by which glucose produces the same effect. A vast amount of evidence has been marshalled against the view that the sulfonylureas affect hepatic glucose output or peripheral glucose utilization except through insulin release, but the question of a direct effect on the liver has not been entirely resolved.[75] There have been suggestions that sulfonylureas affect the plasma factors that influence insulin action (see above) but the significance of the effect to their hypoglycemic action remains to be assessed.[76]

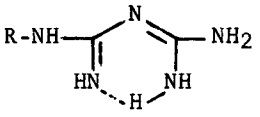
It has long been recognized that the hypoglycemic effect of the sulfonylureas is related to their concentration in the blood.[77] As with many acidic drugs, plasma half life is a key determinant of overall effect[79] and studies of their physiological disposition have proved to be important in guiding their clinical use.[59,78] The metabolism of tolbutamide and chlorpropamide has been reviewed.[74] The metabolism of glycodiazine (plasma half-life, 3.5-4 hr.)[80,81] and tolazamide (plasma half-life, 7 hrs.)[82] has been studied in human subjects.

From the point of view of metabolism, acetohexamide is the most interesting of the sulfonylureas. Although its plasma half-life is short (1.3 hrs.), it is reduced in the body to a metabolite, L(-)-hydroxyhexamide, which is not only more active (2-2.4 times acetohexamide), but is also longer lasting (half-life, 4.6 hrs.).[82,83] The net result is that hypoglycemic activity in the blood, which is due to acetohexamide and the metabolite, persists for about the same length of time as it does with tolbutamide. The fact that the metabolite is at least twice as active as the racemic form of the compound would imply that the D(+) form is essentially inactive, which seems incredible, considering how widely significant activity is found among the sulfonylureas. Unfortunately, synthesis of the compound has not yet been reported. Other metabolites of acetohexamide, which are essentially inactive as hypoglycemic agents, result from hydroxylation in the cyclohexyl group.[83]

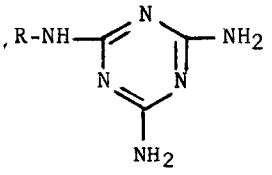
The importance of solubility, and of solution rate, as a function of surface area, to rate of intestinal absorption and hence to the time course of blood levels has been investigated.[66,84,85,86] Proper consideration of these factors in dosage form preparation can be important determinants of overall efficacy.[84]

Biguanides

The biguanides are widely used in the control of diabetes; phenethylbiguanide (phenformin, DBI, Dibotin) in the United States, and, in addition, butylbiguanide (buformin, Silubin) and N,N-dimethylbiguanide (metformin, Glucophage) in Europe. Their principal clinical application is in combination with sulfonylureas for the treatment of patients whose disease is poorly controlled on either agent alone and for smoothing the hypoglycemic response of insulin-dependent brittle diabetics.[87,88] Few new compounds of the class have been reported recently, but a series of aralkylamino, aryloxyalkylamino, and arylthioalkylamino-s-triazines (V) designed from consideration of the structure of the biguanides when written in the cyclic, hydrogen bonded form (IV) has been prepared. The compounds had only weak hypoglycemic activity in rabbits.[89]



IV



V

Pharmacology, Mechanism of Action. The mechanism of biguanide induced hypoglycemia has still not been established. Different investigators cannot yet agree on which of the many effects that have been observed in experimental systems are responsible for their clinical action. The problem has been reviewed and discussed from several points of view.[74,90,91,92,93] It seems clear that glucose utilization by anaerobic glycolysis is increased, the further metabolism of the resulting pyruvate is inhibited, and lactate

therefore accumulates. This implies an acceleration of anaerobic glycolysis resulting from a reduction of oxidative metabolic activity; an effect first described by Pasteur and since known as the Pasteur effect, which Randle[52] now prefers to call "respiratory control". From the work of Passonneau and Lowry,[94] Park[13] and his co-workers and Randle[95] and his associates, the mechanism of this effect is now understood in some detail. A thorough discussion of these concepts is contained in Randle's review.[52]

Phenformin has been shown to inhibit some mitochondrial electron transfer reactions and this has been suggested as its primary site of action.[90] Several objections to this hypothesis have been raised, however. First, the concentrations required to inhibit mitochondrial oxidation *in vitro* are much higher than have ever been observed *in vivo*.[91,92] In view of the frequent disparity between *in vitro* and *in vivo* results and problems of tissue and species sensitivity, this objection is not, of itself, particularly serious. However, a second objection -- that closely related hypoglycemic biguanides show a similar hypoglycemic effect but do not inhibit mitochondrial oxidation, whereas others, which are devoid of hypoglycemic activity, are nevertheless potent inhibitors of mitochondrial electron transfer reactions[97] -- deserves careful consideration. Third, it has been shown that low concentrations of buformin (1-5 μg/ml), which are in the range attained *in vivo*, actually increase the activity of the pentose shunt in adipose tissue and this is cited as evidence that the hypoglycemic activity of the biguanides does *not* involve inhibition of oxidative processes.[91] The conclusion that oxidative metabolism is not inhibited seems untenable, considering that hypoglycemic doses of phenformin invariably cause increases in blood and urinary lactic acid and that pyruvate tolerance is decreased.[98] The buformin effect on adipose tissue is very similar to the effect of epinephrine[99] and, considering the reasonable structural analogy between buformin and the aliphatic pressor amines, may result by a similar mechanism.

One of the most pertinent investigations of the effect of phenformin on metabolism is that of Williamson, Walker and Renold.[100] Using the isolated perfused rat heart, they found substantial increases in glucose uptake and lactate formation and decreased intracellular ATP at phenformin concentrations of 35-50 μg/ml. The authors interpret these findings in the light of current concepts of neucleotide and phosphate feedback control of phosphofructokinase, as the rate controlling step of glycolysis, and further suggest possible inhibition of pyruvate decarboxylation. Like many investigators of the biguanides, Williamson, Walker and Renold discount the relevance of their results to the clinical situation because of the relatively high drug concentrations used. Such differences seem much less important if one considers possible tissue and species sensitivity. The rat is remarkably insensitive to phenformin, showing little hypoglycemic effect at 80 mg/kg[101] while human diabetics respond well at doses of 50-150 mg (1-2 mg/kg).[98] It has been pointed out that an additional component of hypoglycemic action probably results from impairment of the reconversion of lactate, formed in muscle, to glucose in the liver.[90,102] The inhibition of pyruvate decarboxylation mentioned above would accord with the decreased lipogenesis that has been suggested by recent clinical observations,[103,104,105] since reduced availability of acetyl CoA would be expected to impair lipid synthesis. This aspect of the action of the biguanides does not appear to have been studied

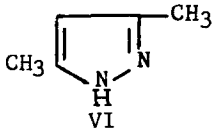
directly. The possibility of a direct action of the biguanides on adenyl cyclase to cause cyclic 3',5'-AMP activation of phosphofructokinase has not apparently been considered but it is an intriguing one. Although cyclic 3',5'-AMP is known to activate mammalian phosphofructokinase, a hormonal effect through adenylcyclase stimulation has not yet been demonstrated.[106,125]

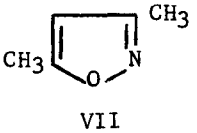
The interesting enigma that the biguanides do not cause hypoglycemia in non-diabetic individuals has received some clarification recently.[107] Glucose turnover studies in non-diabetic human subjects have shown that glucose utilization is markedly increased but that hypoglycemia does not occur, apparently because the increased utilization is offset by a corresponding increase in glucose output from the liver. It seems reasonable to suggest that an effect on hepatic glucose output is the key difference, in this respect, between diabetics and non-diabetics. Hepatic glucose output in diabetics comes mainly from gluconeogenesis, which phenformin inhibits;[102] whereas the hepatic glucose output of non-diabetics, under the conditions of this experiment, would come primarily from glycogenolysis, which phenformin accelerates.[90]

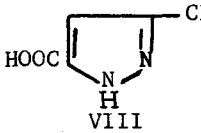
The distribution, metabolism, and excretion of phenformin, metformin and buformin have been investigated.[108,109,110,111] The physiological disposition of the biguanides is typical of that of other strong organic bases. The compounds diffuse from the blood, where concentrations are invariably low and not very persistent, into acidic gastric juice, are found in higher concentrations in tissue than in the blood, and are eliminated efficiently in the urine. The identity of their metabolites has not been established with certainty.

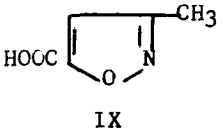
Other Hypoglycemic Compounds

Pyrazoles and Isoxazoles. Since 1963, a series of reports have described two interesting hypoglycemic and lipolysis inhibiting compounds, 3,5-dimethylpyrazole (VI) and 3,5-dimethylisoxazole (VII).[112,113,114,115]


VI


VII


VIII


IX

Both compounds are remarkably potent hypoglycemic agents (50 and 200 times as active as tolbutamide) in glucose primed rats but are much less active in fasted animals. Both are apparently inactive, themselves, but depend for activity on metabolism to 3-methylpyrazole-5-carboxylic acid (VIII) and 3-methylisoxazole-5-carboxylic acid (IX), respectively.[116,117] Although the carboxylic acid is a relatively minor metabolite of dimethylpyrazole (10-15% in the rat), it apparently accounts for all of the activity. The major metabolite, 4-hydroxy-3,5-dimethylpyrazole (70-75% in the rat) shows no hypoglycemic activity.[117] The two carboxylic acids are potent inhibitors of the release of free fatty acids from adipose tissue and the authors make the reasonable suggestion that this is their primary action, from which the hypoglycemic effect results.[118,119] The mechanism by which inhibition of

lipolysis might promote glucose utilization has been discussed extensively by Randle,[48] and these compounds might well be used in further examination of some of his hypotheses. 5-Methylpyrazole-3-carboxylic acid was effective in reducing the plasma free fatty acid levels of fasted human subjects[118] but the concomitant effect, if any, on their blood glucose was not reported. None of a series of pyrazole congeners of 3,5-dimethylpyrazole was apparently more active than the parent compound.[120]

L-Leucine. The subject of leucine induced hypoglycemia, which occurs in certain sensitive individuals, particularly some children with "idiopathic hypoglycemia" and patients with functioning islet cell tumors, has been very ably reviewed recently by Fajans.[121] Addition of L-leucine (12 g/day) to the regimen of sulfonylurea secondary failures resulted in good diabetic control in 8 of 10 patients.[122]

A series of hexahydroindenio[1,2-c]pyrrols and indanamines have been reported to reduce the blood glucose of orally treated rabbits but details that might permit an assessment of their potential as oral antidiabetic agents are lacking.[123]

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Chapter 16. Atherosclerosis

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Introduction. - Atherosclerosis appears to be a "disease of regulation" (I. H. Page) in which a genetically conditioned cardiovascular system is reacting to an ever-changing environment. The ability to regulate this interaction determines in the long run whether atherosclerosis will, or will not, prevail. The disease is a lesion of large and medium-sized arteries characterized by intimal thickening with focal deposits in the intima of yellowish plaques containing lipids, carbohydrates, blood products, fibrous material and calcium. In this constricted artery thrombus formation occurs in the ulcerated atheromatous plaque which results ultimately in occlusion. Serum cholesterol levels and blood pressure are useful predictors of the disease and obesity, smoking, level of physical activity, psycho-social influences, increased triglyceride and carbohydrate metabolism, enhanced blood clotting, hormonal balance and genetics are factors which play a role but their role is as yet unclear. There has evolved the concept that atherosclerosis is a multifaceted disease and that no single cause exists. Two main theories, both of which are susceptible to experimental investigation, exist today. The filtration concept of Aschoff[1] focused attention on disordered lipid metabolism and still constitutes the broadest approach to atherogenesis while the thrombogenic theory proposed by Rokitansky (1844) and revived by Duguid[2] led to increasing interest in intramural clotting mechanisms and their relationship with plasma lipids.

While lipids appear to bear a relationship to human atherosclerosis and associated thrombosis, their involvement still is based on assumption rather than fact. Whether lipids deposited in the vessel come from the blood or are synthesized in the blood vessel is still debated. Increasing attention is being paid the blood vessel, itself. In the atherosclerosis-susceptible White Carneau pigeon enhanced aortic fatty acid and cholesterol ester synthesis is highly correlated with the severity of the disease[3]. In the rat, hypertension increases cholesterol synthesis[4] and cholesterol levels[5] in the aorta as well as in the liver and other tissues.[6] Chapman[7] provides evidence that the force which disrupts an atherosclerotic plaque to form a thrombus proceeds from within the vessel wall toward the lumen. Regardless, by far the greatest effort has been concentrated on reducing blood lipid levels, particularly cholesterol, in the hyperlipemic patient.

Diet. - Epidemiological studies provide a basis for the hope that dietary adjustment may reduce the incidence or at least delay the development of atherosclerosis and coronary heart disease. Preliminary results from the national diet-heart study[8] and the "anti-coronary club"[9] are encouraging and the A.M.A. Council on Foods and Nutrition recommends dietary treatment[21] and prophylaxis against coronary heart disease. The risk of coronary heart disease seems to bear at least some relationship to cholesterol in the blood as shown by the Framingham study.[10] Among dietary agents which influence cholesteremia are total fats,[11] polyunsaturated fats,[12] the ratio of polyunsaturated to saturated fat,[13] cholesterol,[14] amino acid content[15] and carbohydrate.[16] A recent review[17] discusses the relationship of dietary fat and coronary heart disease.

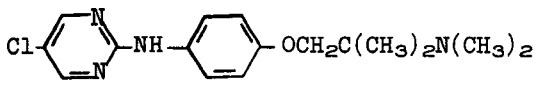
A technique using the isotopic steady state[24a] suggests a limited cholesterol absorption in man and this may be his major protection against a high cholesterol diet[24] since there appears to be no feed-back mechanism in man to compensate for dietary cholesterol intake.[24b] On a high cholesterol diet 60-80% of the serum cholesterol is derived from endogenous sources.[24a] This contrasts with a recent report that the serum level of cholesterol in humans is proportional to the square root of the dietary intake of cholesterol.[25] The rate of synthesis of cholesterol in the small intestine also is independent of cholesterol feeding or fasting.[26] Earlier work with primates showed that they do not respond to dietary cholesterol; however, atherosclerosis can be induced[27] when saturated fats are added to a high cholesterol diet to facilitate absorption. Although cholesterol feeding will produce myocardial infarcts in rabbits, no correlation has been seen in this species between the occurrence of infarcts and the level of cholesterol in the blood.[23]

The fall of serum cholesterol on feeding a diet high in unsaturated fats occurs in the absence of cholesterol excretion changes. This fall in cholesterol levels in the rabbit[28] and in man[29] seems to be due to plasma-tissue redistribution. An alteration in lipoprotein structure results such that equilibrium between plasma and tissue cholesterol pools favors the latter.[30] This correlates with the finding that manipulating the dietary fat intake is not accompanied by a reciprocal change in cholic acid turnover[31] nor is there a consistent relationship between sterol excretion and changes in serum cholesterol concentrations.[32] The hypocholesteremic effect of dietary linoleic acid in the rabbit appears to be proportional to the extent to which linoleic acid is incorporated into plasma cholesterol esters.[34] Measurement of the lipid composition of arterial tissue and atheroma in humans, however, has shown only an increased linoleic acid content in atheroma in those on a diet high in unsaturated fats as compared to controls.[33]

A serum protein deficiency is reported in humans with coronary artery disease.[18] An adequate intake of dietary protein seems to be necessary for effective regression of coronary atherosclerosis in chickens.[19] In chickens on an atherogenic diet, cerebrosides and soy sterols reduce serum cholesterol and this reduction correlates with retarded development of atherosclerosis.[20] It has also been suggested that a substance in soy bean meal will protect rabbits on a high fat diet against atherosclerosis.[22]

Serum Lipids. - Many attempts have been made to reduce serum cholesterol by inhibiting its biosynthesis. Triparanol was introduced some years ago for this purpose but was withdrawn because of severe systemic reactions[37] (lenticular cataracts, alopecia and ichthyosis). It also appears to increase aortic atherosclerosis in chickens[38] and produces congenital malformations in mice and rats.[39] Compounds recently reported, which are similar in many respects to triparanol, include some pyridyl analogs,[40] cyanostilbenes,[41] triarylmethanols[42] and other basic carbinols.[42]

Of some basic ethers of N-hetero-substituted anilines which show cholesterol lowering activity,[43] compound 1 is most active. Three new classes of



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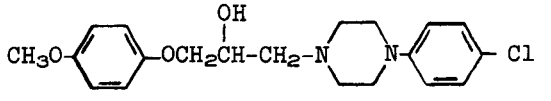

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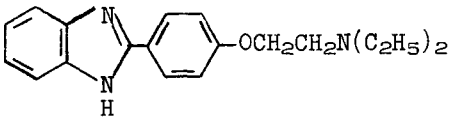

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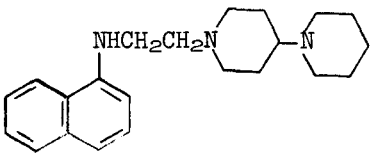

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inhibitors are shown in structures 2 [journal gives name as 4-(2-chlorophenyl)-α-(p-methoxyphenoxymethyl)-1-piperazine], 3 and 4.[44] These compounds (2-4) were shown to accumulate 7-dehydrocholesterol in the plasma and tissues of rats and in this respect are similar to compounds related to AY-9944 reported by Ayerst.[45] 25-Azacholesterol blocks cholesterol synthesis at the desmosterol stage[46] as has also been seen with 20,25-diaza-[47] and 22,25-diazacholesterol.[48] In man 20,25-diazacholesterol lowers blood cholesterol but toxic effects are numerous.[47b] Myatonia and Keratoderma have been reported[49] and in each instance serum desmosterol was elevated and cholesterol was reduced at the time these toxic effects became evident. Some 4-azacholestenes[50] have also been reported to inhibit cholesterol synthesis. Cyclization of squalene is inhibited by tolbutamide[51] while phenethylbiguanide inhibits conversion of farnesyl pyrophosphate to squalene.[51]

Phenyl- and biphenyl-substituted acids have been studied extensively for their cholesterol lowering activity.[52] Activity is reported also with biphenyl ether-substituted acids.[52c] The agent of most interest in this area remains p-chlorophenoxyisobutyric acid (CPIB, Clofibrate, atromid S). The reader is directed to the Atromid symposium[53] for background information. In bovine vascular tissue *in vitro*[54], in rats[55] and in man[56] CPIB inhibits cholesterol biosynthesis, apparently between mevalonic acid and isopentenyl pyrophosphate.[55] It was again confirmed that CPIB alone is as active as the combination of CPIB and androsterone (Atromid)[57] and that it lowers triglycerides to a greater extent than cholesterol.[57b] It also markedly decreases low density lipoprotein (a suggested mode of action for CPIB)[58] and reduces the glyceride content of high density lipoprotein. CPIB is reported to decrease the β/α lipoprotein ratio[59] and increase uric acid excretion.[60] The norepinephrine-induced rise in free fatty acid levels is unaffected by CPIB in humans.[61] The effect of CPIB on the fibrinolytic system is variable[62] but does not appear to be significant.[63]

Prospective studies of large groups has verified that the risk of myocardial infarctions is substantially greater in cigarette smokers than in non-smokers[64] and this appears to be mediated *via* the nicotine-induced release of epinephrine.[65] Nicotine, in dogs, has been shown to raise cholesterol[66] and blood sugar[67] and this may be related to the elevation of free fatty acid levels.[66] Caffeine raises free fatty acid levels[68] and it is suggested that coffee intake can be associated with heart disease.[64b]

Agents which will regulate free fatty acid levels are of interest both in diabetes and atherosclerosis. 3,5-Dimethylisoxazole,[69] 3,5-dimethylpyrazole[70] and its metabolite, 5-methylpyrazole-3-carboxylic acid[71] dramatically lower fatty acids and blood sugar in animals. Infusion of norepinephrine produces a rapid rise in free fatty acid and in plasma triglycerides of low density lipoprotein.[72] Nicotinic acid blocks this norepinephrine-induced rise in free fatty acid[73,61] and lowers free fatty acid[74] and triglyceride[75] levels in the blood. It is also reported to reduce the cholesterol content of various tissues in cholesterol-fed

rabbits.[76] In humans it produces abnormal carbohydrate metabolism.[77] Aluminum nicotinate is effective as a hypocholesteremic agent and appears to be better tolerated than nicotinic acid.[78] A series of methoxamine analogs[79] and a variety of antidepressants[80] are also reported to block the lipolytic effects of epinephrine. Single injections of insulin cause acute falls in free fatty acids in man[82] while in another study a combination of potassium, glucose and insulin has been used to treat myocardial infarction with some benefit.[81]

Various agents reported to lower cholesterol in animals are chondroitin sulfate A,[83] vanadium,[84] a mitochondrial fraction from starved rat liver[85] and in man, 6-azauridine,[85a] p-aminosalicylic acid[86] and a relative of heparin, a sulfated polyanion SP54.[87] Insulin is reported to decrease the cholesterol content of blood and aorta while increasing the level in liver and adrenals[87a] in cholesterol-fed rabbits.

In principle, feeding substances which remove bile acids from the intestine and which prevent their reabsorption should increase bile acid excretion and speed up cholesterol degradation. This is, indeed, possible with agents such as ferric chloride,[88] aluminum hydroxide[89] and with dietary saponins.[90] The bile acid-binding anion-exchange resin MK-135 (Cholestyramine)[91] shows marked lowering of serum cholesterol in the chicken, rabbit,[92] dog[93] and man[94] and has utility in relieving pruritis associated with biliary cirrhosis.[95] Steatorrhea is a problem with this drug[97] but a report indicates that when medium chain triglycerides are substituted for the long chain triglycerides in the diet this is controlled.[98] Metamucil, an oral hydrophilic colloid, is also reported to lower serum cholesterol.[99]

β-Sitosterol[100] plays a role in inhibiting absorption of cholesterol. The antibiotics neomycin, N-methylated neomycin, but not streptomycin or N-acetylated neomycin, reduce cholesterol in the chick,[101] presumably by interfering with absorption processes. Paramomycin also lowers serum cholesterol while reducing bacterial flora.[102]

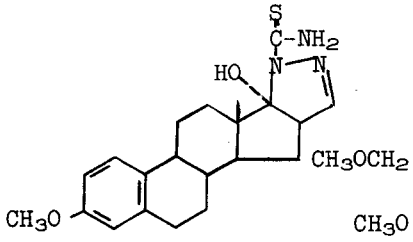
The role of hyperglyceridemia in atherosclerosis is still cloudy[103] but it appears that the triglyceride level is no more useful than cholesterol levels in identifying subjects with ischemic heart disease.[104] Patients with elevated triglycerides also have a high incidence of elevated fasting blood sugars and elevated free fatty acids[105] and thus have an increased risk of myocardial infarction and latent diabetes. It is perhaps significant that in the rat utilization of fat for energy accelerates cholesterol biosynthesis.[35] The development of some varieties of hypertriglyceridemia is accompanied by a corresponding increase in the turnover of palmitate but not linoleate. This may represent increased synthesis of lipid from carbohydrate.[106] Insulin decreases the plasma levels of glyceride in patients with hypertriglyceridemia.[108] Protamine sulfate, an inhibitor of lipoprotein lipase, inhibits this action. CPIB appears to be the best agent to date for lowering elevated triglyceride levels.[57b]

Hormones. - Interest in estrogens stems from reports that coronary artery disease is a rarity among premenopausal women[109] and that it is much greater in castrated women than in those having undergone hysterectomy but retain their ovaries,[109b] although this finding is not without its critics.[110] On the other side, castrated men are less prone to develop coronary disease than normal men.[111]

Estrogens in humans increase α-lipoprotein (rich in phospholipid P) levels but their effect on β-lipoproteins (rich in cholesterol C) is variable. Thus,

the C/P ratio will decrease but there may or may not be a drop in cholesterol levels.[112] Lecithin appears to be the only major phospholipid fraction which is increased.[113] Triglyceride levels are also increased by estrogen administration.[114] Estrogens protect the coronary arteries but not the aorta of the chicken.[115] Experiments in rats had like effects but rabbits failed to show benefit from estrogens.[116] Efficacy in humans still remains a will-of-the-wisp. Significant evidence of their prophylactic value against myocardial infarction has not as yet been demonstrated.[117] Mental functioning in patients with cardiovascular and cerebrovascular disease is reported to improve with estrogen treatment.[129]

Since the side effects of estrogen in the male are a problem, much effort has been devoted to the search for compounds having the lipid profile of an estrogen but devoid of its feminizing properties.[118] Among recent compounds studied are a series of 1-methyl estrogens,[119] 13-alkyl estrogens[120] and a series of 3-alkyloxy-17,17-(or 16,16)ethylenedioxy estratrienes.[121] Compounds 5[122] and 6[123] are reported to show a possible split between their feminizing and lipid properties. On the possibility that a metabolite is responsible for



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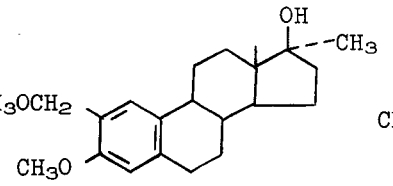

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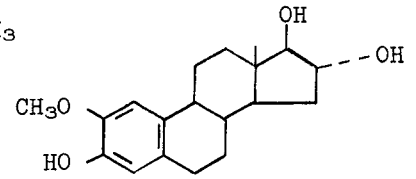

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the lipid effects of estrogens, a series of 2-hydroxy- and 2-methoxy-estradiols and their 16-oxygenated analogs was investigated. Compound 7 is reported to have a large split between the properties in question.[124]

Conjugated equine estrogens increase blood platelet levels[125] during lipemia following its administration but oral contraceptives have little effect, if any, on blood coagulation or fibrinolysis[126] (including platelet adhesiveness) except perhaps to increase factor VII levels.[127] Estrogen therapy increases plasma hydrocortisone apparently by increasing transcortin levels.[128] Gonadoptropins increase side-chain cleavage of cholesterol but not 20α -hydroxy cholesterol indicating that 20α -hydroxylation is stimulated by gonadotropins.[130] This is in line with the recent report that estrogen may decrease cholesterol by increasing the turnover rate, and therefore, the catabolism of cholesterol.[131] The fact that it is not active orally stimulated some effort into finding an androsterone analog which is active orally. 3α-Methoxy-17-methyl-5α -androstan-17β-ol is reported to lower cholesterol levels[134] and enhance excretion of cholesterol in the bile of rats.[135] Methalone, an anabolic steroid, also decreases the level of cholesterol in man.[136]

Cortisone increases levels of triglyceride, phospholipid and cholesterol in rats but does not alter intestinal absorption or the rate of incorporation of acetate into cholesterol.[137] In cholesterol fed rabbits adrenal hypertrophy precedes deposition of cholesterol and it is suggested that the adrenal gland

may act as a regulator intervening in the process responsible for atherosclerosis.[138] In man, hyperlipemia has been seen in acute adrenocortical insufficiency and successfully treated with hydrocortisone.[139] The use of adrenocorticotropic gel has given encouraging results in some cases of acute myocardial infarction.[140]

The hypothyroid state is associated with an elevated plasma cholesterol level while the hyperthyroid state is associated with low cholesterol levels in plasma.[141] It was again pointed out that in a large number of patients hospitalized with acute myocardial infarction the serum PBI levels are lower than normal.[142] The effectiveness of thyroactive substances in lowering serum cholesterol and in reducing atherogenesis has been amply demonstrated in various animal species[141] but their clinical use remains restricted because of their action on basal metabolic rate, and in particular, on oxygen consumption of the myocardium and aggravation of angina.[143] Therefore, interest is focussed on drugs whose effect on lipid metabolism is accentuated relative to their hypermetabolic effect. Many thyroxine analogs have been made, some of which are reported to have a desirable "split" in the two activities.[144] A comprehensive review of this subject has been published recently.[145]

The thyroxine analogs of most interest at the present time are D-thyroxine (D-T_4) and D-triiodothyronine (D-T_3). A controversy still exists as to whether there is any separation of lipid and calorigenic effects of these two agents as compared to thyroxine.[146] D-T_3 is claimed to lower serum lipids in patients with essential hyperlipemia but showed no effect in normolipemic controls.[147] As with D-T_4 it also appears to show a worsening of angina or arrhythmia in humans.[148] Some more recent thyroxine-like compounds which have been studied extensively include D-trichlorothyronine,[149] 4'-methoxy-3,3'-5-triiodothyroacetic acid[150] and a series of thyroalkanols[151] which also appeared to show a "split" in animals.

Thyroidectomy in rats has no effect on oxidation of either labeled cholesterol or sodium octanoate. Preliminary nuclear changes in cholesterol take place at about the same rate regardless of the thyroid state. This and other data are consistent with the earlier hypothesis that the effect of the thyroid on cholesterol metabolism may be mediated at the level of nuclear hydroxylation of the steroid.[153] Increased thyroid levels decreases the production of estriol from estradiol and increases production of 2-methoxyestrone. A recent study[154] shows that 2-hydroxyestrone becomes the major metabolite in subjects with high thyroid levels and that in myxedema 2-hydroxyestrone levels are diminished. The production of androsterone diminishes in hypothyroidism and it has been postulated that this produces increased lipid levels.[132] These complicated endocrine interactions make a study of thyroid activity difficult and its effect on lipid metabolism remains to be clarified.

Thrombosis and Fibrinolysis. - Interest in the thrombogenic theory of atherosclerosis proposed by von Rokitansky and revived by the studies of Duguid[155] has an experimental basis. Artificial production of fibrin deposits or thrombi on vessels will give rise to intimal thickening and eventually atherosclerosis.[156] Fibrin-like material has been demonstrated in atherosclerotic lesions.[157] localization of atheromatus changes may be related to mechanical forces which lead to the formation of thrombi at stress points.[158] The influence of lipids in the pathogenesis of atherosclerosis might be felt not only in their deposition in the intimal lesion but also in their clot-promoting effects[159] (phospholipids

and free fatty acids can produce platelet aggregation[160]) and their capacity to inhibit fibrinolysis.[161,159b] Chemical attack in this area has been at inhibiting the initial event, platelet aggregation, and at dissolution of the thrombus (fibrinolysis). For background the reader is directed to a comprehensive review on platelets and atherosclerosis[162] and the recent workshop on fibrinolysis.[163]

Adenosine diphosphate (ADP) will aggregate platelets in vitro and this aggregation can be prevented by enzymatic conversion of the ADP to ATP.[164] This induced aggregation requires a complexable cation and fibrinogen.[165] The formation of thrombin and ADP a few seconds after blood is shed has been demonstrated in vivo[166] and thus both of these agents are present before the platelets are available. Epinephrine also induces platelet aggregation[165] and thrombosis in rats[167] along with norepinephrine and serotonin. Pronethalol, a β-blocker, blocks the epinephrine-induced platelet rise and aggregation.[168] β-Blocking agents have also been found useful in aleviating angina of effort[169] and propranalol is reported to reduce the mortality from acute myocardial infarction.[170]

Initial reports[171] that linolenic acid caused a decrease in platelet adhesiveness and reduced the incidence of experimental thrombosis have been controverted by the same authors[172] and by others.[173] Among materials which are reported to reduce platelet adhesiveness are Dipyramidole,[174] a series of adenosine analogs,[175] histamine[176] and methyl mercuric nitrate.[177] M.A.O. inhibitors were shown not to protect against experimental thrombosis,[178] in disagreement with an earlier report. Dextran appears to have some clinical utility in thrombus inhibition[179] and has been successfully used in severe ischemia.[180] It appears to inhibit platelet factor III by coating the platelet.[182]

The role of anticoagulants in the treatment of myocardial infarction is unclear. Experiments with rabbits,[183] dogs[184] and rats[185] have shown that heparin will prevent experimentally induced thrombosis. Conflicting reports suggest that anticoagulants may prolong the time for platelet aggregation[186] but heparin in man showed no consistent changes in platelet economy or platelet adhesiveness[187] and only a feeble effect in protecting against thrombotic occlusion after arterial reconstruction.[188] Increased sensitivity to heparin was seen in patients following acute myocardial infarction.[189] In patients who suffered a myocardial infarction while on anticoagulant therapy, there was a decrease in anticoagulant levels as compared to those with no myocardial infarction.[190] In patients with coronary heart disease the hematocrit and whole blood viscosity is significantly higher than in normals.[191] The role played by the lipid-clearing activity of heparin is still under investigation.[192] Long term anticoagulant therapy after myocardial infarction, using heparin[193] and the coumarins,[194] appears to be beneficial and it has been recommended[195] that this form of treatment be used routinely.

In a cephalin fraction which stimulated the clotting mechanism, it was found that phosphatidyl-ethanolamine was the only component which showed consistent high activity.[196] In a study of patients with myocardial infarction these authors[196] showed that, although the total cephalin fraction was similar to that in the controls, the patients had cephalin with higher clotting activity and markedly higher oleic acid content. Insulin produces acceleration of blood clotting and decreases fibrinolytic activity[198] in dogs, attributed to the sympathico-adrenal mechanisms for restoration of normal glycemia since it is

blocked by dihydroergotamine. Progestational agents are also reported to produce hypercoagulability in women.[199]

Thrombolytic therapy has recently been reviewed.[200] The enzymes streptokinase,[201] staphylokinase[202] and urokinase[203,201c] appear to have utility in acute thromboembolic vascular diseases. CA-7 (a fibrinolytic enzyme from aspergillus oryzae) has produced lysis of arterial thrombi in dogs.[204] Phenformin[205] (DBI) and Metformin[206] have shown promise as fibrinolytic agents which may be useful in the prophylaxis of arterial occlusion. The authors have suggested that phenformin acts like a corticoid and have indeed shown it to be beneficial in rheumatoid arthritis.[205] An earlier report[207] that atromid has fibrinolytic activity was contradicted recently.[208] A large number of vasoactive drugs, both hyper- and hypotensive are reported to have fibrinolytic activity which is related to their vasoactive changes.[209] Complamin (xanthinol nicotinate) has been shown to possess fibrinolytic activity in man[210] but the patients very quickly develop a tolerance to the drug.

Psycho-Social Factors. - One group of investigators[211] has undertaken the study of behavior patterns and their relationship to coronary artery disease. They find that "Behavior Pattern A" (restless, competitive attitude) men have higher cholesterol levels,[212] faster clotting times[212] and have a much higher incidence of arcus senilis and coronary artery disease.[213] The same relationship was found in women.[214] Individuals harboring pattern A excrete more epinephrine during their working day than those in pattern B (relatively non-ambitious, non-aggressive).[215] Subjects of pattern A have also shown a high incidence of blood sludging after a fatty meal whether the fats were saturated or unsaturated, while sludging was virtually absent in non-coronary prone individuals.[218] Similar relationships occur when animals are stressed.[216] It is implied that social factors may account for the low incidence of myocardial infarction in Roseto, Pa.[217] The inhabitants are strikingly obese, eat considerable quantities of animal fat and their serum lipid patterns do not differ from those found in the Framingham study,[10a] yet they have a very low incidence of myocardial infarcts as compared to those in the Framingham study.

Summary. - There is clearly no unified concept of atherogenesis. Knowledge in the field is chaotic and treatment, or prevention, is still a matter of opinion.

The preferred method for inhibiting atherogenesis is still control of the diet. Of interest is the evidence which shows that substituting unsaturated for saturated fats in the diet causes a plasma-tissue redistribution rather than inhibiting synthesis or increasing excretion rates of cholesterol. Dietary cholesterol seems to play only a minor role, if any, in the development of human atherosclerosis. Drugs which lower serum lipids are still scarce. Efficacy of CPIB on long term treatment of the disease remains to be seen. Cholestyramine, D-T_4, estrogens and nicotinic acid have their champions and will find special usage. Increased activity has focussed attention on platelet adhesiveness, although no promising agent has emerged as yet. The fibrinolytic enzymes still are the best agents for fibrinolysis therapy. A start has been made in unraveling psycho-social factors and relating behavior with serum lipid patterns and proneness to coronary heart disease. Research in atherosclerosis must still be considered in its infancy. With increasing interest by medical scientists and by governmental agencies, progress at an accelerated rate can be expected.

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Chapter 17. Non-steroidal Hormones and Their Antagonists

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INTRODUCTION

The non-steroidal hormones considered in this section are composed primarily of amino acid derivatives. Structures of the simpler substances, such as the thyroid hormones and oxytocin and vasopressin have been known for some time. Recent advances center around structural variations of known hormones, structural elucidation of new hormones, and efforts of the initial syntheses of hormones whose structures have been proposed on the basis of degradation studies. The compounds selected for review are limited to those whose structures are known, or have been proposed, and in which significant recent progress has been made in the synthesis of the hormone or of its analogs.

A hormone is generally considered to be a discrete chemical substance, produced in a gland, secreted into the body fluids, and producing a specific effect on the activities of other organs. The "glandular hormones" clearly belong to this class. Recently, a number of peptides with intense pharmacological activities have been found to be generated by specific enzyme catalyzed hydrolysis of plasma proteins; these have been called "tissue hormones." An additional miscellaneous group related by peptide nature and pharmacological properties have thus far been isolated only from non-mammalian sources.

HORMONES OF THE PANCREAS

Insulin.--Recombination experiments of synthetic and natural A and B chains of insulin have been reported from German, Chinese and American groups. The Aachen group described a synthesis of the A-chain of sheep insulin and its combination with the native B-chain to produce biological and chemical properties like those of insulin.[1] Synthesis of the B-chain and condensation with the native sheep A-chain produced about 0.65% insulin activity.[2] Combination of synthetic A and B chains of sheep insulin produced biological activity 0.2 to 1.0% that of native insulin.[3] An improved recombination of bovine insulin was reported to produce 44% insulin-like activity using a 1:1 mixture of the preoxidized A-chain and the reduced B-chain.[4]

The Shanghai-Peking groups reported combinations of synthetic B-chain with the natural A-chain of bovine insulin, and of the synthetic A-chain with the natural B-chain to give crystalline products with 2-4% of the activity of natural insulin;[5,6] slight biological activity was obtained initially by combining synthetic A and B chains. Improved synthesis of A and B chains,[7] using azide condensations at the final stages of synthesis to preclude racemization, and use of excess of the A-chain and air oxidation at pH 10.6[8] led to a synthetic bovine insulin with initial activity of 1.2-2.5% that of natural insulin based on the protein concentration present. Purification by solvent extraction and crystallization in a citrate buffer containing acetone and zinc acetate yielded a crystalline product identical in crystal shape and biological activity (mouse convulsion and rabbit hypoglycemia) with natural bovine insulin. Chromatographic and isotope dilution studies with C-14 labeled synthetic insulin

confirmed identity with the natural protein.[9]

The Pittsburgh group has followed earlier work on the combination of synthetic A-chain and natural B-chain of sheep insulin (0.5-1.2% activity),[10] with synthesis of the B-chain and combination of this with the natural A-chain to produce insulin-like activity equivalent to that produced by recombination of the natural A and B chains.[11] Considerable activity was also found when synthetic B-chain was combined with a partially purified preparation of synthetic A-chain.

The B-chain of human insulin has been prepared as the S-sulfonate and combined with the natural A-chain of bovine insulin to produce 4-8% the activity of crystalline bovine insulin.[12] Synthesis of human A-chain, and combination with synthetic human B-chain and with natural bovine B-chain yielded 2% and 8% insulin activity by the mouse convulsion method.[13]

The combined results of the German, Chinese and American groups indicate that totally synthetic preparation of pure human insulin is possible with presently available techniques, and that the tools are available for important studies of insulin analogs.

Glucagon.--The amino acid sequence of the glycogenolytic pancreatic hormone, glucagon, was determined by Bromer and co-workers in 1957. Partial syntheses of the linear peptide, which contains 29 amino acids, have been reported by Schröder[14], and by Wünsch[15], but biological activities have not yet been reported for these glucagon-related peptides, and the complete synthesis of glucagon is not yet reported.

HORMONES OF THE PITUITARY

The Melanocyte-Stimulating Hormones (MSH) and Adrenocorticotropic Hormones (ACTH).--Presence of the same amino acid sequence from 4 through 10 may account for certain common biological properties of MSH and ACTH. β-MSH from various species possess additional N-terminal amino acids, and vary in the nature of amino acids 1-3 in the α-MSH structure.

Melanocyte-Stimulating Activity.--The ability to produce skin darkening in amphibia has been associated with the pentapeptide sequence 6-10, His- Phe-Arg-Try-Gly, common to both ACTH and MSH. This requirement has now been shortened to the tetrapeptide, His-Phe-Arg-Try (6-9), which has been shown to possess both melanocyte-stimulating and lipolytic activity at levels comparable to those of the pentapeptide.[16] Alteration in the (6-10) pentapeptide sequence has produced one of the few examples of inhibition by close analogs in the peptide field. The citrulline analog, His-Phe-Cit-Try-Gly elicits no MSH-activity, but inhibits the activity of α-MSH.[17] Substitution of D-amino acids in the pentapeptide produces compounds which range in activity from MSH-like to antagonists. The all-D isomer, D-His-D-Phe-D-Arg-D-Try-Gly showed no melanocyte-stimulating or lipolytic activity, but lightened the color of a specimen predarkened by either the all-L pentapeptide, or by synthetic α-MSH. Premixture of equal amounts of all-L and all-D isomers blocked the darkening effect on the isolated frog skin.[18] The L-D-D-L and L-L-L-D (6-10) peptide sequences

retained MSH-activity, while the D-D-D-L, L-D-D-D and D-L-L-L analogs were inactive, or weak antagonists.[19]

The influence of chain length on MSH-activity has been summarized by Schwyzer.[20] Synthetic analogs of ACTH, lengthened from the N-terminal to the C-terminal, show initial melanocyte-stimulating activity at the decapeptide (1-10), although the nonapeptide (1-9) was not investigated. Activity increases markedly at the tridecapeptide amide stage, and activity remains constant with further elongation of the C-terminal end. Acetylation of the amino group of the N-terminal serine residue generally enhances activity 10 to 100-fold in the series.

α-MSH Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Try-Gly-Lys-Pro-Val-NH_2

ACTH H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Try-Gly-Lys-Pro-Val-
1 2 3 4 5 6 7 8 9 10 11 12 13

Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Try-Pro-
14 15 16 17 18 19 20 21 22 23 24

Asp-Gly-Glu-Ala-Glu-Asp-Ser-Ala-Glu(NH_2) (Beef)

Ala-Gly-Glu-Asp-Asp-Glu-Ala-Ser-Glu(NH_2) Ala-Phe-Pro-Leu-Glu-Phe (Sheep)
25 26 27 28 29 30 31 32 33 34 35 36 37 38 39

Asp-Gly-Ala-Glu-Asp-Glu(NH_2)-Leu-Ala-Glu (Pig)

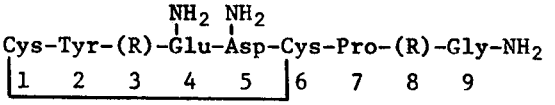
Adrenocorticotropic Activity.--The total synthesis of β-corticotropin, with the amino acid sequence characteristic of the porcine species, has now been reported in detail.[21] The techniques developed have aided the synthesis of smaller fragments which have been used to relate chain length and ACTH activity.[20] Corticotropin activity is associated with the presence of a free terminal amino group. From the amino end, threshold activity is found with the 1-13 tridecapeptide amide, corresponding to the free amino form of α-MSH. Four to five amino acid residues after MSH activity has reached its plateau, ACTH activity sets in strongly, reaching an almost constant value at 20-24 residues, then climbing to twice this value at the 39 residues of ACTH. The hexacosapeptide corresponding to the first 26 residues of bovine ACTH has been reported to possess adrenal- and melanocyte-stimulating activity comparable to the native hormone on a molar basis.[22]

Ramachandran, Chung and Li[23] have pointed out the possible significance of the concentration of all the basic amino acid residues of ACTH in the region between positions 5 and 22, with a solid core (Lys-Lys-Arg-Arg) in positions 15-18. The positive charge contributed by the basic amino acids is considered possibly to contribute to receptor binding with a negatively charged surface. The ten-fold increase in potency from the heptadecapeptide (1-17) to the nonadecapeptide (1-19) may be attributed to the additional arginine at 18, which may contribute a positive charge to receptor binding.

Further enhancement of activity by conversion of the terminal carboxyl group to an amide in this series,[24] could be due to the decrease in negative charge contributed by the carboxylate ion, which would interfere with associative interactions at a negatively charged receptor. The approximately equal activity for the octadecapaptide and nonadecapeptide amides was felt to support this concept, [23] since both contained the same core of basic amino acids. The acidic residues concentrated in the carboxyl terminal part of the molecule are suggested as playing a secondary role in enhancing receptor binding at a positively charged site. Since the amino terminal end is most sensitive to alteration, and many functional amino acid residues implicated in the active sites of proteolytic enzymes are present, this region is considered to be the active site of the hormones, oriented by the binding affinities of more distant basic and acidic centers.

The importance of the basic amino acids has been further indicated by the essential loss in steroidogenic ACTH-like activity with the hexadecapeptide, $\alpha^{1-14,\ 17-18}$-Gly^1-ACTH, and heptadecapeptide, $\alpha^{1-14,\ 16-18}$-Gly^1-ACTH-18-NH_2 in which lysine residues have been removed from the normal 1-18 sequence of ACTH analogs.[25] Synthesis of the active analog, 17,18-diornithyl-β-corticotropin-(1-24)-tetracosapeptide adds further evidence for the important role of basic amino acids in the 15-18 region of the ACTH molecule.[26]

Oxytocin and Vasopressin.--Boissonnas[27] and Law[28] have published recent comprehensive reviews of structural analogs of these hormones of the posterior lobe of the pituitary.



	R_3	R_8
Oxytocin:	Ile	Leu
Lysine-vasopressin;	Phe	Lys
Arginine-vasopressin:	Phe	Arg

The following data extends from the structure-activity correlations of Law.[28] The question of the importance for hormonal activity of the 20-membered disulfide ring has been investigated further. Analogs of arginine- and lysine-vasopressin and their 1-deamino derivatives in which the ring was open and the S-atoms replaced by H-atoms (e.g., Ala^1-Ala^6-Arg^8-vasopressin) were devoid of hormonal-like or antagonistic activity.[29] Additional methylene groups in the ring eliminated activity, as shown by the inactivity of the 21-membered ring compound, 4-β-alanine-oxytocin, when compared with the moderately active 20-membered ring analog 4-glycine-oxytocin,[30] and of 1-γ-mercaptobutyric acid oxytocin, compared with the highly potent deamino-oxytocin (1-β-mercaptopropionic acid oxytocin).[31] Although the intact 20-membered ring requirement has thus far been upheld, isosteric replacement of the ring atoms is possible with retention of activity. Cyclic analogs of oxytocin in which one of the S-atoms of the disulfide bridge was replaced by a methylene group,[32] or by a selenium

atom (6-seleno-oxytocin and deamino-6-seleno-oxytocin)[33] showed significant hormonal activity. Synthesis of diseleno-oxytocin has been reported, but final purification and biological evaluation was not carried out.[34] The stereochemical relationship between the ring and the side-chain is important, as shown by the loss in oxytocic activity with both 1-hemi-D-cystine- and 6-hemi-D-cystine-oxytocin.[35]

All-D-oxytocin has been prepared and shown to lack either hormone-like or inhibiting effects.[36] Replacement of L-Tyr^2 by D-Tyr^2 in oxytocin and its 1-deamino analog produced analogs which retained significant, although slight, oxytocin-like activities as well as an inhibiting effect on oxytocin.[37] The analogs L-Dab^8- and D-Dab^8-vasopressin[38], containing the shorter basic side-chain derived from α,γ-diaminobutyric acid, showed significant pressor and antidiuretic activities. The D-analog showed selective antidiuretic activity relative to its pressor and uterotonic effects. The configuration of the basic amino acid at the 8-position seems more important for pressor, rather than antidiuretic action, as shown by the selective antidiuretic effects of D-Arg^8- and D-Lys^8-vasopressin.[39]

Alteration of side chains has aided the definition of structural requirements for activity, and has produced analogs with selective and prolonged effects. Both vasopressin and oxytocin show enhancement of activity in the 1-deamino derivatives.[40,41] Systematic replacement by hydrogen of other functional groups in amino acid side chains of oxytocin has shown that the phenolic hydroxyl group is not vital, but does contribute significantly to activity. The carboxamido groups are essential at 5 (asparagine) and 9 (glycinamide), but not critical at 4 (glutamine), since glutamine may be replaced by α-aminobutyric acid with retention of significant biological activity.[41] Removal of the carboxy terminal NH_2- group alone (9-deamido oxytocin) leads to loss of activity.[42]

Acylation of the amino end of vasopressin (Gly-Cys^1-vasopressin) resulted in a partial reduction in activity, but a three-fold prolongation of effects.[43] Preparation of further extended-chain analogs of oxytocin, including Pro-Cys^1-, Phe-Cys^1-, D-Leu-Cys^1-, Leu-Leu-Cys^1-, and Gly-Gly-Cys^1- oxytocins has been reported.[44] The observation of oxytocin-antagonistic properties for analogs of oxytocin with O-ethyltyrosine, p-methylphenylalanine, and p-ethylphenylalanine replacing tyrosine,[45] has been extended by synthesis of Gly-Cys^1-Tyr(Me)^2-vasopressin. This chain-lengthened and functionally modified analog showed a small but prolonged antidiuretic effect, and a relatively strong inhibition of the pressor activity of lysine-vasopressin at a molar ratio of analog to hormone of 25:1. Complete and long-lasting inhibition was produced at a ratio of 200:1. Complete inhibition of the uterotonic effect of oxytocin on the isolated rat uterus was obtained at a ratio of 300:1.[43] N-Carbamyl-O-carbamyl-oxytocin showed no effect alone on the isolated rat uterus, but at an analog to hormone ratio of 50:1, produced complete inhibition of oxytocin administered one minute later. The mono-acylated derivatives, N-carbamyl- and O-carbamyl-oxytocin showed slight oxytocic (0.05 and 0.1%), and no inhibitory effects on the rat uterus.[46]

The requirements of position 8 have been studied in detail. An aliphatic side chain is important for oxytocic activity, since Gly^8-oxytocin was inactive,

while an ethyl, or even methyl group present in But^8-oxytocin and Ala^8-oxytocin conferred a high degree of activity.[47] The importance of the basicity of the amino acid residue at position 8 and the phenolic group at position 2 for vasopressin-like activity was shown with analogs combining variations at the 2 and 8 positions. Phe^2-Orn^8-vasopressin and Phe^2-Orn^8-oxytocin showed selective pressor activity [48]while desamino^1-Arg^8- and desamino^1-Phe^2-Arg^8-vasopressins showed an enhanced antidiuretic effect.[49] Syntheses of desamino^1-Orn^8- and desamino^1-Phe^2-Orn^8-vasopressins and oxytocins has also been described.[50] Thialysine^8-vasopressin, an isostere containing sulfur in place of a methylene group of the lysine side chain, showed antidiuretic activity greater than that of Lys^8-vasopressin, with less pressor activity. Therefore, factors other than base strength appear to be involved in the role of the side chain in the 8 position.[51]

Reaction of oxytocin with acetone under mild conditions to produce an inactive derivative has been noted.[52] Reaction is presumed to occur with the free amino group, since deamino^1-oxytocin is not deactivated under these conditions. Specific tritiation of oxytocin by catalytic deiodination has been reported.[53]

HORMONES OF THE STOMACH

Gastrin.--Heptadecapeptide amides gastrin I and gastrin II isolated in 1964 from hog antral mucosa, are believed to be related to the hormone released during digestion, which stimulates gastric secretions. On a molar basis, gastrin II is some 500 times more effective than histamine in stimulating gastric secretion. Gastrin II differs from gastrin I only in being the sulfate ester derivative of the tyrosine residue.

Pyr-Gly-Pro-Tyr-Met-(Glu)$_5$-Ala-Tyr-Gly-Try-Met-Asp-Phe-NH_2

Gastrin I

Only the C-terminal tetrapeptide amide sequence, Try-Met-Asp-Phe-NH_2 was found to be required to produce the full range of physiological effects of the natural hormones.[54] Structure-activity studies[55,56] in the active C-terminal tetrapeptide sequence led to the following observations: (a) all N-acyl derivatives of the tetrapeptide are active, (b) substitution of all, or part of the L-amino acids by the D-isomers leads to virtual loss of activity, (c) replacement of tryptophane by other amino acids results in loss of activity, although 4-, 5-, and 6-methyl tryptophanes are highly active, (d) methionine may be replaced by norleucine or ethionine with retention of activity, byt all changes in the aspartic acid position led to inactive compounds, (e) O-methyl tyrosine may replace phenylalanine, (f) the terminal amide cannot be converted to the methyl ester, the free acid, or a dialkylamide without loss in activity.

TISSUE HORMONES

Bradykinin, Kallidin and Methionyl-lysyl-bradykinin.--Bradykinin and its N-terminal analogs are formed by enzymatic degradation of inactive precursors present in the α_2-globulin fraction of plasma.

R-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
1 2 3 4 5 6 7 8 9

Bradykinin, R = H Kallidin, R = Lys Methionyl-lysyl-bradykinin, R = Met-Lys

From a comprehensive survey of analogs in 1964,[57] it was concluded that the importance of individual amino acids for biological activity was in the order, 7,8>1>9>5>4>2>3>6. The Pro^7 and Phe^8 residues appear to be most essential for biological activity. The terminal arginine residues (1,9) may be replaced with basic amino acids, but neutral amino acids produce a loss of activity. The aromatic residue in position 5 is essential. Exchange of Gly^4 and Pro^2- residues causes medium loss of activity, while the Pro^3 and Ser^6- residues are relatively insensitive to change.

The importance of the free carboxyl end of bradykinin, and of other active peptides for full biological activity, has been emphasized.[58] Variations in the nature and configuration of the peptide chain have led to less active or inactive analogs.

Retrobradykinin,[59] containing the reverse sequence of amino acids, all-D-bradykinin[58,60], and all-D-retrobradykinin[60] showed neither bradykinin-like nor antagonistic activity. Replacement of either or both of the L-arginine residues with D-arginine resulted in loss of activity, and no production of inhibitory effect.[61] Introduction of ester in place of amide links between residues 3-4 and 5-6, in the depsipeptides glycolyl-4- and glycolyl-6-bradykinin produced analogs with 0.5 to 1% activity.[62] The inactivity of the cyclic decapeptide, Gly^7-cyclokallidin,[63] as compared with the active linear Gly^7-kallidin, further demonstrated the importance of conformation for biological activity.

A number of octa- and nonapeptide analogs containing threonine in place of serine were prepared as potential antagonists.[64] Tests on rat uteri showed variable and unpredictable mimetic and antagonistic response; only bradykinin-like effects were seen with the isolated guinea pig ileum or rat duodenum. The solid-phase synthesis of bradykinin[65] has been extended to provide a number of additional bradykinin analogs. Among these, replacement of both phenylalanine residues with O-methyl tyrosine yielded an antagonist at low concentrations, but bradykinin-like activity at higher concentrations.[66] To date, no bradykinin antagonistic peptide has been reported which is free of bradykinin-like activity at high dose levels. Extension of the N-terminal end of bradykinin generally leads to little change in activity.[67] Met-Lys-bradykinin is about 1/3 as active on isolated muscle preparations, but 2-3 times as active as bradykinin in lowering rabbit blood pressure. The methionine sulfoxide retains the activity of Met-Lys-bradykinin, while the sulfone is only about 1/10 as active. N-terminal-extended bradykinin analogs are generally highly active, with (Lys-Lys-Arg^1)- and (Lys-Lys-Lys-Arg^1)-bradykinin being 8-10 times as potent as bradykinin in lowering rabbit blood pressure, while being less active in producing contractions of isolated smooth muscle.

Dihydrochlorprothixene and a related analog have been reported to act as competitive inhibitors of bradykinin on isolated guinea pig preparations. Cyproheptadine acted as a non-competitive inhibitor.[68]

Angiotensin.--Angiotension I, a relatively inactive decapeptide produced by the action of renin on an α_2-globulin blood fraction, is converted to the active octapeptide, angiotensin-II by the enzymatic removal of a C-terminal dipeptide,

His-Leu. Species differences provide two equiactive angiotensins, containing valine or isoleucine in the 5-position. The composition of human angiotensin is not yet established.

Asp-Arg-Val-Tyr-Ile (Val)-His-Pro-Phe
1 2 3 4 5 6 7 8

The kinetics of the renin-substrate reaction have been studied employing a synthetic tetradecapeptide renin substrate.[69]

Earlier studies which provided structural relationships relative to smooth muscle-stimulating or pressor effects have been summarized by Law.[28] For significant activity, the carboxyl and phenyl groups of Phe^8 and phenolic hydroxyl group of Tyr^4 appear to be essential. Loss of activity on photolysis and decreased activity of analogs containing 6-substituents supported the essential role of the imidazole ring of His [6]. The low activity of the Ala^7 analog indicated the importance of Pro^7. Substitution of leucine for valine or isoleucine in the 5-position resulted in a four-fold decrease in activity, but leucine could replace Val with complete retention of activity, indicating a selective influence of side chain branching at position 5. A low degree of specificity was generally indicated for Asp^1 and Arg^2.

Aromatic residues (4,6,8).--Using Asp $(NH_2)^1$-Val^5-angiotensin-II as the parent compound, Schröder[70] has replaced the His^6 residue with phenylalanine and lysine, obtaining activities of 1% and 0.1%, respectively. This provides further evidence for the importance of histidine, with aromatic character apparently being more important than base strength. A surprising loss in activity occurred when O-methyl tyrosine replaced Tyr^4(0.2%), considering the relatively strong activity reported for the Phe^4 analog (10%). Exchange of hydroxyl groups between positions 4 and 8 with Asp $(NH_2)^1$-Phe^4-Val^5-Tyr^8-angiotensin-II led to inactivity. Apparently the phenolic character of Tyr^4 and aromatic character of Phe^8 are the most important features, as shown by the 10% activity of the di-tyrosyl analog, Asp $(NH_2)^1$-Val^5-Tyr^8-angiotension-II. The closely related Ile^5-Tyr^8-angiotensin-II has been reported as only slightly less active than Ile^5-angiotensin-II.[71] Increased separation between the Tyr^4 and Phe^8 residues, by exchange of the Val^3 and Tyr^4residues, led to loss of activity.[70]

Aliphatic residues (3,5,7).--Tyrosine in place of Val^3 resulted in 12% activity;[70] hydroxyproline in place of Pro^7 reduced activity to 7%.

Acidic and basic residues (1,2).--Variations of Asp^1 have yielded analogs with heightened and prolonged activities. The isomeric α-D-, β-L-, and β-D-Asp^1-Val^5-angiotensins-II, by comparison with natural α-L-Asp^1-Val^5-angiotensin-II and its Asp^1-β-amide, show an increase in activity of about 50%, and a two to three-fold prolongation of pressor activity in the rat.[72] This has been attributed to a decrease in the rate of degradation of the modified angiotensins by aminopeptidases. Desamino-Val^5-angiotensin-II showed a 50% decrease, but prolongation of activity. This series has been extended by Schröder[73] who showed that the D-Asp^1-, Glu^1-, Glu $(NH_2)^1$- and Pyroglu^1-Val^5-angiotensin-II analogs all showed about 50% enhancement of pressor activity over that of Val^5-angiotensin-II.

The activities reported for the Lys^2 and Val^2 analogs of Asp $(NH_2)^1$-Val^5-angiotensin-II (10% and 5%) added to the conclusion that the arginine at position 2 is not essential for activity.[74] In a study mainly involving 2-8 heptapeptide analogs, X-Val-Tyr-Ile-His-Pro-Phe, Havinga[75,76] has concluded that the features of the arginine moiety contributing to activity are the positive charge or the capacity to hydrogen bond of the guanidinium or ammonium groups forming part of X in active peptides. Heptapeptides with D-amino acids in the X-position were more active than the corresponding L-compounds. However, 1-8 octapeptides with D-amino acids in the 2-position were less active than their L-counterparts.

All-D-Asp$(NH_2)^1$-Val^5-angiotensin-II has been synthesized as a potential antagonist, but showed neither angiotensin-like nor antagonistic effects in both smooth muscle-stimulation and pressor tests.[77,78]

The solid-phase method of peptide synthesis has been used to prepare Ile^5-, Asp$(NH_2)^1$-Ile^5-, and β-Asp^1-Ile^5-angiotensins-II.[79]

The problem of the secondary structure of angiotensin in solution is unresolved. Thin-film dialysis studies indicate a coiled or compact form of low axial ratio.[80] Biological experiments involving inhibition of smooth muscle responses to angiotensin by urea and amino acids, indicate that the simple compounds produce either randomization of an organized angiotensin structure, or produce inhibition by direct interaction with the receptor.[81]

MISCELLANEOUS PHYSIOLOGICALLY ACTIVE PEPTIDES

Eledoisin and Physalaemin.--The powerful vasodilating hypotensive undecapeptide eledoisin was isolated in 1962 from the salivary glands of a mollusc. In 1964 the four-fold more potent related undecapeptide, physalaemin, was isolated from the skin of a South American amphibian.

Eledoisin H-Pyr-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2

Physalaemin H-Pyr-Ala-Asp-Pro-Asp(5-NH_2)-Lys-Phe-Tyr-Gly-Leu-Met-NH_2
(1 2 3 4 5 6 7 8 9 10 11)

Studies of numerous analogs have shown that similar structure-activity relationships exist in both compounds. The C-terminal amino acids (6-11) are highly sensitive to alteration, while the N-terminal residues (1-5) are relatively insensitive.[82] A minimum of five amino acid residues at the C-terminal end (7-11) is needed for appreciable activity, while activity increases sharply with the hexapeptide (6-11) and higher analogs. The hexapeptide (6-11) related to eledoisin retains 30% activity in lowering blood pressure in the rabbit, while the heptapeptide (5-11) is essentially as active as eledoisin.[83] The corresponding physalaemin hexapeptide (6-11) is 2.2 to 3.8 times as active as eledoisin, and at least half as active as physalaemin in its hypotensive effect in the dog.[84] In a series of Asp$(NH_2)^5$-eledoisin analogs, maximal hypotensive activity in the rabbit was reached in the octa to decapeptide range,[85] while maximal hypotensive activity in the dog was shown at the C-terminal nonapeptide (3-11)

related to eledoisin.[86]

Increased activity has been shown in eledoisin analogs with higher thioalkyl groups replacing the thiomethyl group of methionine, while alkylcysteinamide residues, with one less methylene group, or other amino acids, drastically reduce activity.[87]

The all-D isomer of the highly active hexapeptide analog of eledoisin (6-11) was devoid of either eledoisin-like or antagonistic activity. Systematic replacement of individual L-amino acids by D-amino acids in the active heptapeptide (5-11) resulted in essential loss in activity, except for the D-Ala^6 analog, which retained 30% of the activity of the parent heptapeptide, in lowering blood pressure in the rabbit.[88]

With few exceptions, both physalaemin and its close analogs are relatively inactive in producing contractions of the rat uterus, as compared with eledoisin, in doses equiactive in reducing blood pressure.[84]

THYROID HORMONES

Thyroxine and Triiodothyronine.--Recent contributions to studies relating structure and activity may be discussed in terms of the structure of thyroxine.

Phenolic hydroxyl.--Thyroxine-like activity found for 4'-deoxy-3,5-diiodothyronines, and inactivity for corresponding 4'-methyl analogs was attributed to a probable metabolic introduction of the hydroxyl group to produce the active molecule.[89]

Outer ring substituents.--The ability of alkyl or aryl groups to replace halogen atoms on the phenolic ring with retention, or even enhancement, of biological activity, has been confirmed. Relative to L-thyroxine (100), substituted 3,5-diiodothyronines showed the following antigoitrogenic activities in the rat: L-3'-iodo (775), DL-3'-ethyl (340), L-3'-isopropyl (1250), L-3'-isopropyl-5'-iodo (440), and L-3'-isobutyl (60).[90] Barker has noted similar potent thyroxine-like effects in oxygen consumption and heart rate tests on rats for thyroxine analogs with outer ring alkyl substituents, or bearing a fused phenyl ring (3,5-diiodonaphthyronine).[89] In these tests, as well as a test measuring inhibition of thyrotropic hormone release,[91] the 3'-isopropyl analog was the most potent tested. 3'-Tertiary butyl-3,5-diiodo-L-thyronine showed equal activity to L-thyroxine in the tadpole metamorphosis assay,[92] and about twice the activity of L-thyroxine in the rat antigoiter assay.[93] Further examples of decreased activity for outer ring 3',5'-disubstituted analogs, as compared with the corresponding 3'-monosubstituted compound were presented.[89] A long standing apparent inconsistency in this regard was resolved by the demonstration that the 3'-chloro analog was more active than 3',5'-dichloro-3,5-diiodo-L-thyronine.[94] The importance for biological activity of a distal positioning in space relative to the inner ring for the outer ring substituent has been emphasized by further examples.[89] The role of ring substituents in establishing skewed conformation for the diphenyl ether nucleus has been studied on thyroxine analogs using NMR techniques.[95]

Ether linkage.--Three biphenyl analogs of tetra- and triiodothyroacetic acid, and of thyroxine were found to be essentially inactive in the heart rate test,[89] indicating a requirement for the ether linkage. However, the biphenyl analog, 3,5-diiodo-4-(3',5'-dimethyl-4'-hydroxylphenyl) phenylalanine was reported to be equiactive to the corresponding diphenyl ether, and about 1.5 times as active as thyroxine in inducing metamorphosis in the immersed tadpole.[96] Iodinated 2-methyl-3-carboxy-5-hydroxybenzofurans, compounds lacking the diphenyl ether nucleus, and previously reported active in the tadpole metamorphosis assay, were inactive in the heart rate test.[89]

Inner ring substituents.--The 3,5-dibromo analog of DL-thyroxine approached the activity of thyroxine in the rat oxygen consumption test,[89] indicating the lack of a requirement for iodination on the alanine-bearing ring. Thyronines mono-iodinated, or lacking iodine on the inner ring were inactive in oxygen consumption tests.

Side chains.--Thyroxine analogs with alcoholic side chains of varying lengths have been prepared by reduction of the corresponding acids.[97] Among these, 3,5-diiodo-3',5'-dimethyl thyroethanol was reported to show in the rat a greater dissociation between hypocholesterolemic and calorigenic effects than either L- or D-thyroxine. A number of reports of synthesis and biological activities of α-methyl thyronines have appeared.[98-100] α-Methyl-DL-thyroxine[99] showed weak thyroxine-like activity in antigoitrogenic, cholesterol-lowering, and heart weight assays, and inactivity as a thyroxine antagonist in the anti-goiter test. α-Methyl-DL-triiodothyronine and its D- and L-isomers were reported to be more active in the hypocholesteremic test than DL-α-methyl thyroxine.[100]

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Chapter 18. Reproduction

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Introduction

There is every reason to believe that History will record the year 1955 as one of those rare turning points in the Life of Man for it was then that the first steps toward effective family planning and self-control of population pressures were made; the elements of oral contraception began to take shape.[1] True, it was only a beginning. But it was a beginning built on a solid foundation, and it offered hope: Hope to young couples not ready to have children; hope to older couples who could ill afford to expand their families; hope to children where each new mouth to be fed would threaten further their slim chance for survival. And it offered hope to nations already straining every resource in a futile effort to maintain an already inadequate level of existance.

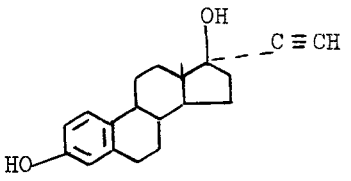
At a time when the laws of exponential growth were beginning to thrust populationsinto critical periods of survival, the development of oral contraception was long overdue...and almost too late! Progesterone(3) the hormone of the corpus luteum and fundamental precursor of most steroid hormones, had been known since 1928.[2] It had been isolated and synthesized by several research groups by 1934.[3] By 1937 it was known to block the estrus cycle in the rat[4] and to block ovulation in the rabbit.[5] Eight more years were to pass before hormonal therapy was to be considered seriously as a means of effecting birth control[6] and still ten more years before the right steroids, in the hands of some people with imagination....and a plan, produced results of extraordinary importance. Thus Rock, Pincus, and Garcia came to report that ovulation could be inhibited by administering any of three 19-norsteroids orally between day 5 and day 25 of the menstrual cycle.[1]

Like so many important events in history, the outcome may have been determined by a fortunate coincidence. Both norethynodrel (8) and norethindrone (6b) (which were selected for further study) were contaminated with biologically significant amounts of an estrogenic impurity[7] while norethandrolone (6a), a weak androgen, capable of producing serious liver dysfunction,[8] was not pursued.

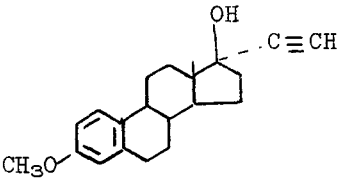
The corpus luteum and its secretory product progesterone, are generally held responsible for maintaining pregnancy and blocking ovulation during the extended period of gestation. A number of synthetic progestins are also recognized to be effective ovulation inhibitors in animals. It is therefore not surprising that investigators in the field considered the 19-norsteroids first studied to be simply "progestins."[7] Unfortunately the term continues to be applied erroneously to oral contraceptives in general, whether containing estrogen as an impurity or as the major ingredient in terms of biological effect. Actually most contraceptives marketed or under study involve progestin <u>plus</u> estrogen, in combination or sequentially.

Available Steroid Contraceptives

Currently, all contraceptives marketed contain either ethinyl estradiol (1) or its 3-methyl ether, mestranol (2). The former is a highly potent oral estrogen for which a large body of clinical experience exists. The latter was a contaminant in early samples of norethynodrel and norethindrone and is generally added to those products in an amount required to maintain a fixed composition. It is believed that ethinyl-estradiol may be slightly more potent than mestranol since 0.05 mg. of the former and 0.08 mg. of the latter are needed daily to inhibit ovulation in women.[9]

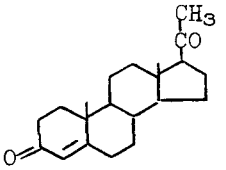


1 Ethinylestradiol

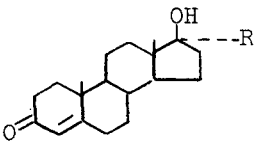


2 Mestranol

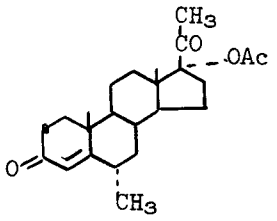
To date, all known progestins are derived formally either from the structure of the male hormone testosterone (4a) or from the female hormone progesterone (3). Among the former, ethisterone (4b), or 17-ethinyltestosterone, although the only available oral progestin for many years,[10] has apparently not been used in oral contraception. It has weak androgenic properties.[11] The 19-nor analog, norethindrone (6b), is also weakly androgenic, both in animal studies[12,13] and in the clinic.[14-17] Its 17-acetate is generally similar in activity.[9] Norethandrolone (6a), first marketed as an anabolic agent to promote weight gain and nitrogen retention, also exhibits androgenic properties and can produce serious hepatotoxicity over a prolonged period at high dosage.[8] Norethynodrel (8) and norethisterone acetate are readily converted to norethindrone (6b) in vivo[14,18-21] but the former is said to exhibit more estrogenic than androgenic character.[16,22] High potency was also encountered in ethynodiol diacetate (10) wherein the 3-ketone was reduced to an alcohol[23-25] and in lynestrenol (12), an analog of 6b in which the 3-ketone has been removed.[26] Numerous related analogs have been prepared and studied, including haloethynyl and trifluoropropynyl derivatives of 19-nortestosterone,[27,28] 17-chloroethynyl-17β-hydroxyestr-4,9,11-trien-3-one,[29] 7α-methyl-17-ethinyl-19-nortestosterone,[30] 6α,21-dimethyl-17-ethinyltestosterone (14, Dimethisterone)[31] and synthetic 13β-ethyl-17α-ethinyl-17β-hydroxygon-4-en-3-one.[32]



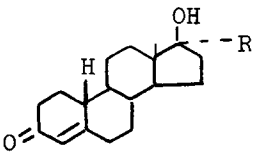
3 Progesterone



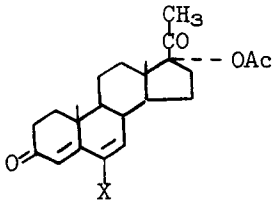
4a R = H, Testosterone
4b R = C≡CH, Ethisterone



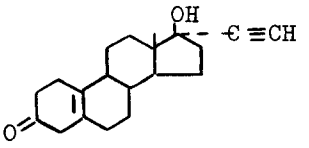
5 Medroxyprogesterone acetate



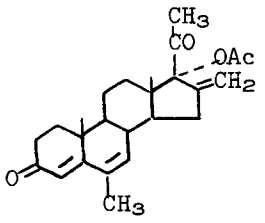
6a R = C_2H_5 Norethandrolone
6b R = C≡CH Norethindrone



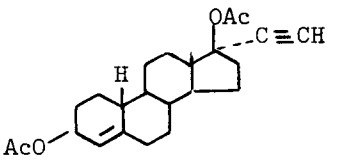
7a X = Cl Chlormadinone
7b X = CH_3 Megestrol acetate



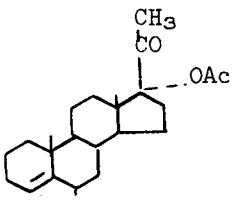
8 Norethynodrel



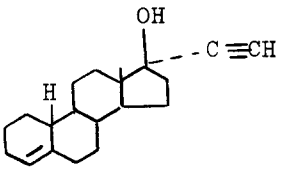
9 Melengestrol acetate



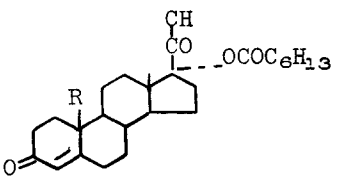
10 Ethynodiol diacetate



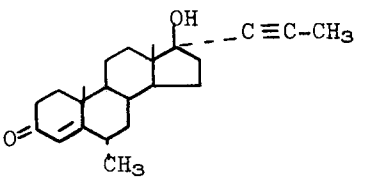
11 DMAP



12 Lynestrenol



13a R = CH_3 Hydroxyprogesterone caproate
13b R = H



14 Dimethisterone

A powerful stimulus to the development of orally effective analogs of progesterone was the recognition of substantial oral activity in 17α-acetoxyprogesterone.[28] While this material was more potent than ethisterone, it was not widely studied because far more potent analogs were rapidly synthesized. Of these, the 6α-methyl derivative, (medroxyprogesterone acetate (5),prepared almost simultaneously in several laboratories,[33-35] found wide clinical use. Unlike the 19-nortestosterone derivatives, it has not been associated in clinical use with virilization of the fetus (in contrast to its effects in lower species) nor with hepatic dysfunction.[36,39] It is one of the few progestins which can be used either orally or by injection. By the latter route, it forms a microcrystalline depot from which drug is available for prolonged activity[40] of up to six months from a single injection.[41]

Introduction of a Δ^6-bond produces megestrol acetate (7b).[34] increasing potency slightly[42] and altering the metabolic pathway.[43-45] The corresponding 6-chloro analog, chlormadinone (7a),[46,47] is also a potent progestin used with mestranol in a sequential formulation. Other closely related 17α-acetoxyprogesterone analogs of interest include the 16-methylene compound, melengestrol acetate (9)[48] and the 3-desoxy compound DMAP (11).[49]

The 17-acetate group can be replaced with a 17-alkyl group without loss of activity, both in 6,17α-dimethyl-6-dehydroprogesterone[50] and in its corresponding 3-hydroxy analog.[51] Similarly, the acetophenonide of 16α,17α-dihydroxyprogesterone is an effective progestin and has been studied in a parenteral contraceptive formulation.[52]

It has been estimated that at least seven million women in the United States are currently using oral contraceptives. For most, the mechanism of action appears to be suppression of the mid-cycle release of LH, preventing ovulation. In a small percentage of subjects, indirect evidence for ovulation has been obtained. The combined action of estrogen and progestin in the combination is thought to produce an endometrium unfavorable for implantation and a cervical mucous barrier difficult for sperm to penetrate.[53] In the sequential method, efficacy depends principally on the inhibition of ovulation during the period when estrogen is administered. Recently, low doses of progesterone alone have been reported to provide a measure of contraceptive efficacy.[54] Further reports will be awaited with interest.

Side effects of the steroid combinations vary with the individual subject and the preparation and may include breast tenderness, weight gain, breakthrough bleeding, amenorrhea, flatulence, tiredness, nausea.....many of the symptoms of early pregnancy. Thromboembolism has not been clearly related to use of oral contraceptives but occasional liver dysfunction, especially in some populations, may be related.

In addition to the steroids used for contraception in the female, a number of synthetic hormones resembling the synthetic estrogens but exhibiting antiestrogenic activity in animals have been studied. These include clomiphene (MRL-41),[55] which is capable of stimulating ovulation in some women[56,57] U-11100A,[58] and a number of closely related analogs.

In the male as in the female, inhibition of fertility can be produced artificially at many stages. Jackson has reviewed the effects of agents in the male (especially as studied in the male rat), and has concluded that while there are many opportunities for pharmacological control, interference with hormonal mechanisms has relatively little prospect for success.[59] Research in this field can be complicated by the relatively long period of treatment and study generally required to observe changes in spermatogenesis. Some agents, however, like methylene dimethanesulfonate (15) affect sperm in the final stages of development and, when used in experimental animals at appropriate dosage, can produce prompt development of infertility and rapid post-treatment recovery.[60] Related methanesulfonates interfere with multiple stages of spermatogenesis, suggesting that selective control at a most appropriate stage may be possible.[61]

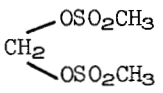
Certain bacteriostatic nitrofurans (16)[62] and thiophenes (17)[63] were also found to interfere with spermatogenesis in the male, blocking development at the primary spermatocyte stage. Similar results, which were reversible were also obtained with bis-(dichloroacetyl)diamines (18).[64-66] These materials, which were originally studied as amoebacidal agents, were relatively well tolerated in man,[67] except for an 'Antabuse-like' effect. Another potent agent which blocks spermatogenesis at the primary spermatocyte stage is the dinitropyrrole ORF-1616 (19)[68] which is effective when given once a month.[69]

Hormonal agents, both steroidal and non-steroidal, have also been found capable of blocking spermatogenesis. Many exert their antifertility effects through gonadotropin inhibition. When used for this purpose, estrogens have the obvious drawback of producing feminization and loss of libido. Androgens have been long known temporarily to suppress spermatogenesis and in selected cases, to produce a rebound in sperm production.[70-72]

A number of progestins have recently been evaluated in the male, including norethandrolone (6a) and 17α-hydroxyprogesterone caproate (13a).[72] These agents inhibit Leydig cell function and gonadal hormone production. Medroxyprogesterone acetate (5)[33] a potent progestin effective both orally and parenterally, was observed to produce a marked decrease in sperm count and motility lasting up to six months when given as a single 1000 mg injection to male volunteers.[73] The long duration of action of this progestin is the result of the formation of an intramuscular depot of slowly released drug when administered as a microcrystalline aqueous suspension by the intramuscular route.

Clomiphene (MRL-41), an agent which can induce ovulation under some conditions in women, inhibits testicular and accessory gland weights in the male rat to the range seen after hypophysectomy.[57,74] However, when administered orally to young men in doses of 50-200 mg/day, clomiphene stimulated Leydig cell function.[72] A biologically similar dihydronaphthalene derivative, U-11100A (21),[58] was found to inhibit spermatogenesis at a dose of 0.5 mg/kg in the rabbit.[75]

The hydrazine derivative ICI 33828 (22) also is effective in both male and female rats and in other species and has been studied in man.[74,76]



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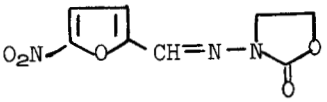

Nitrofurantoin
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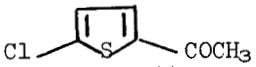

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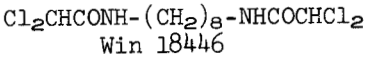

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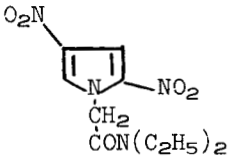

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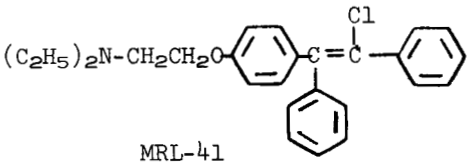
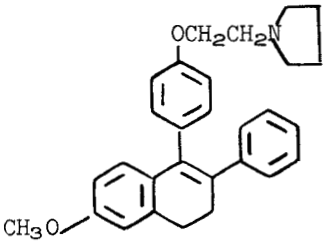
(19)



MRL-41
(20)



U-11100A
(21)

ICI 33828
(22)

Among nutritional factors affecting fertility, the role of Vitamin E and A have recently been reviewed.[77,78]

The rapidly developing knowledge of immunological phenomena as applied to fertility and infertility has also been reviewed[79,80] and can confidently be expected to provide important new approaches during the next decade.

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Chapter 19. Steroid Hormones and their Antagonists

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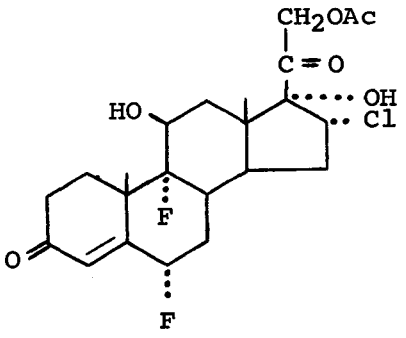
A catalog of biologically active steroids which have appeared in the scientific and patent literature through 1963 has been published.[1,2] The following is a review of the more active steroids and steroid-hormone antagonists which have been published recently.

Corticoids

Corticoids have as their primary clinical utility the control of inflammation. Recent reviews of this and other related activities of corticoids have been published.[3-7] Several new 16α-halocorticoids have been synthesized,[8] of which 16α-chloro-6α,9α-difluoroprednisolone 21-acetate (1) is 1,100 times as potent as hydrocortisone in inhibiting granuloma formation in rats when administered subcutaneously. Several 17-desoxycorticosteroids, including 16α-methyl-1-dehydrocorticosterone (2)[9] and their 6α-fluoro-[10] and 9α-fluoro-derivatives (2a and 2b),[9] have been prepared and their corticoid activity measured.



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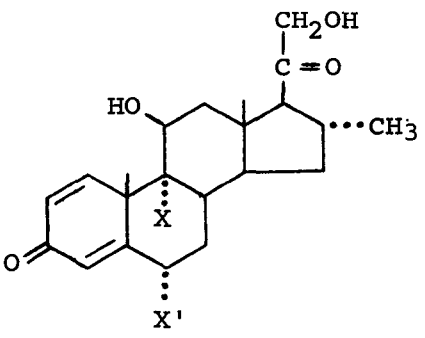


2 X = X' = H
2a X = H, X' = F
2b X = F, X' = H

Comparison of the thymolytic activity of some new 21-chlorosteroids with the corresponding 21-hydroxy compounds has shown[11] the danger of projecting the biological relationship of one series of steroid compounds into another.

The mechanism of the anti-inflammatory action of steroid hormones remains obscure; however, the regulatory effect of corticoids on metabolism may be an important factor.[12-14] Additional reports[15-17] have been made on the thymolytic action of corticoids.

The antiglucocorticoid activity of 1-dehydrotestololactone on cortisone acetate without inhibition of antigranuloma or thymolytic activity has been reported.[6]

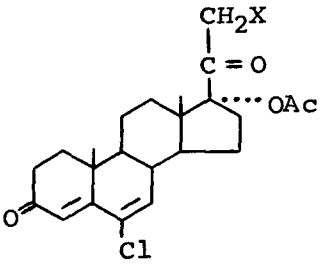
Reviews on antialdosterone compounds have been published.[18,19] There is as yet no known potent naturally-occurring, sodium-losing steroid.[20]

Progestational Compounds

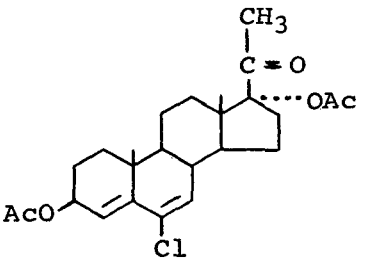
Progestational agents have been tested for many biological criteria, several of which are translatable into clinical utility.[21-23] Among these criteria are the proliferation of the uterine endometrium, maintenance of pregnancy in the overectomized animal and inhibition of ovulation. In general, synthetic compounds are initially evaluated in the Clauberg[24] or the modified Clauberg (MacPhail)[25] assays. It is difficult to compare activities reported from many laboratories because of variation in method, route of administration and reference standards.

A number of new progestationally active compounds have been reported. The 21-fluoro-derivative 3a[26] of chlormadinone (3) is approximately 1.5 times as active orally as 3 in the Clauberg assay, and the 3β-acetoxy compound 4[27] is 100 times norethindione by oral administration. The 7-dehydro-derivative 5a of medroxyprogesterone (5) has been synthesized[28] and has an improved ovulation inhibiting/progestational index. The totally synthetic 4,9,11-trienes 6 and 6a have been found[29] to be orally as active as is progesterone administered subcutaneously. A number of retrosteroids have also been prepared,[30] the most active being the 6-fluoro-6-dehydro compound 7. The 17α-vinyl compound 8 has been compared[31] to norethynodrel (9) with regard to progestational activity. Both compounds demonstrate the same spectrum of activities, although 8 has slightly higher progestational activity and lower estrogenic activity than norethynodrel.

Salts of steroids have been found to have progestational activity on oral administration. These include the 3-ammonium sulfate 10 and the 3-pyridinium sulfate 11.[32] The 3β-(1-pyrrolidyl)-derivative 12[33] and the tricyclic compound 13[34] also have progestational activity.



3 X = H
3a X = F



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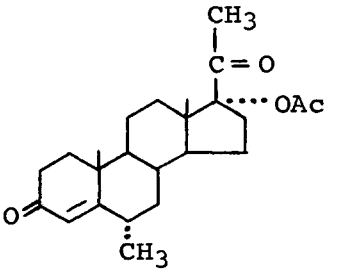
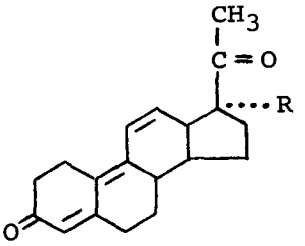
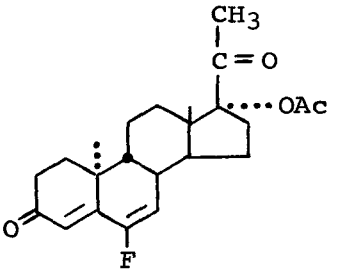


5
5a Δ^7



6 R = H
6a R = CH_3



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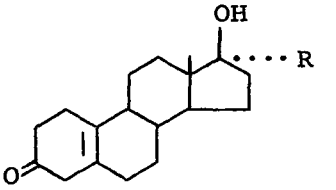
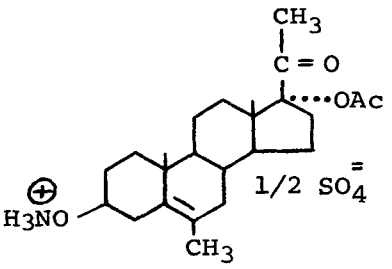


8 R = $CH=CH_2$
9 R = $C\equiv CH$



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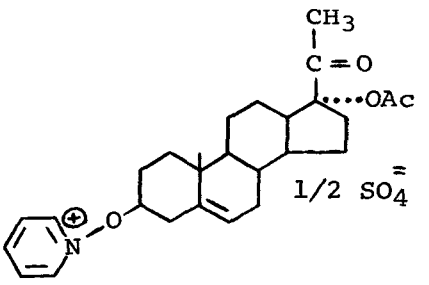

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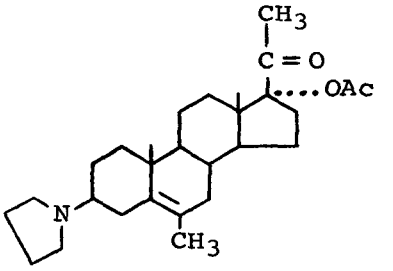
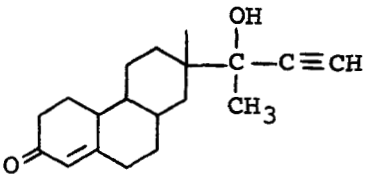


12

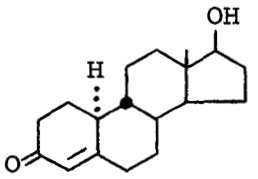


13

Androgens

Androgenic steroids, in addition to having an effect on the growth and secretion of sex organs and accessory sex organs, produce effects on other tissues. These include bone, muscle, blood, lymphatic system, hair and skin. Recent reviews[23,35,36] on this subject, as well as on the clinical utility of androgens, have been published.

Several new structural types have been shown to have androgenic activity. These include the 19-nor-retrosteroid 14,[37] the tricyclic acids 15 and 16,[38] the 17-pyrrolidinium tosylate 17[39] and 6-methylandrost-5-ene-3β-ol-17-one (18).[40]



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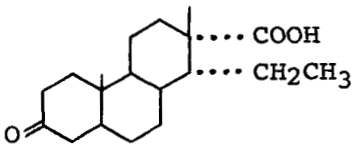
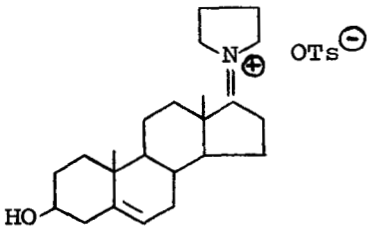


15 Δ^4
16 Δ^1



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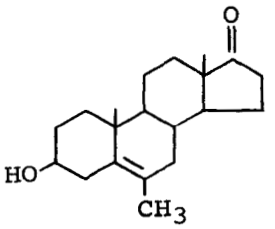
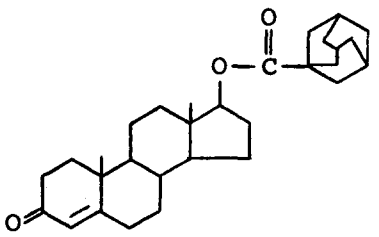


18

A number of compounds have been synthesized in an attempt to separate the anabolic activity of androgens from their virilizing properties. These include testosterone 17β-adamantate (19),[41] the retrosteroids 20[42] and 21,[43] the 3,3-azosteroid 22,[44] the 2,3-furazan 23,[45] 1α,7α,17α-trimethyltestosterone 24[46] and substituted 17β-tetrahydropyran-2-yl ethers,[47] all of which have good dissociation quotients.



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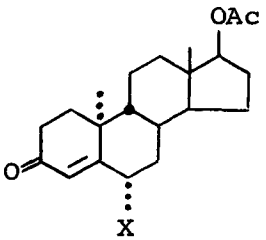
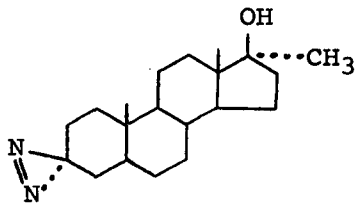


20 X=Cl, $\Delta^{1,6}$
21 X=F



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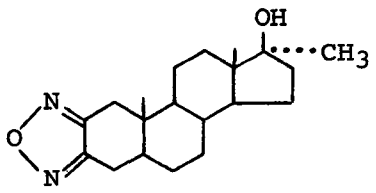

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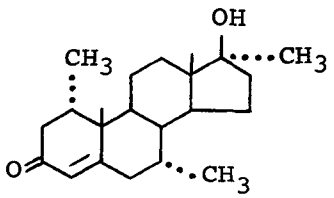
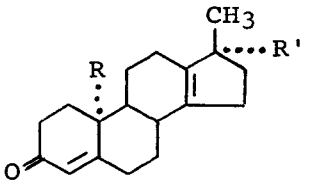


24

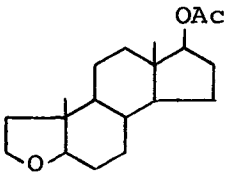
The biological properties of antiandrogenic steroids as well as their possible clinical utility has been reviewed.[35,48,49] As a rule, progestational compounds as well as a number of weak androgenic compounds have shown antiandrogenic properties. Synthetic efforts have been directed to separate hormonal properties from antiandrogenic activity. Among the recently synthesized compounds which have antiandrogenic activity are 17,17-dimethyl-18-norandrosta-4,13-diene-3-one (25),[50] the 17α-ethyl-19-nor- compound 26,[51] 3β-selenosteroids[52] and the tricyclic compound 13.[34] The

A-nor steroids 27[53] and 28[54] continue to show antiandrogenic activity which has been characteristic for this series, and the B-nor steroid 29[55,56] shows similar activity. The 16α-bromo-17-ketal 30[55] had significant antiandrogenic activity in the testosterone-stimulated mouse.

An example of a potent progestational compound which has strong antiandrogenic properties is the 1,2-methylene compound 31.[48,57]



25 R = R' = CH_3
26 R = H, R' = C_2H_5



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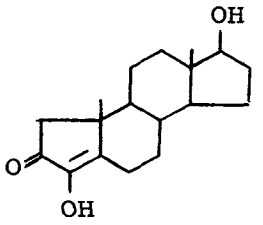

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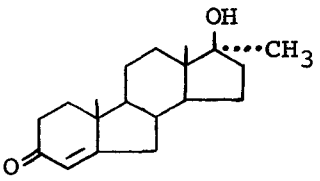

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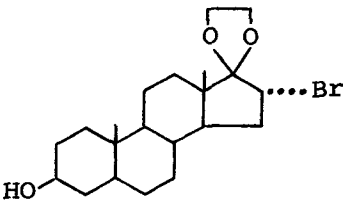

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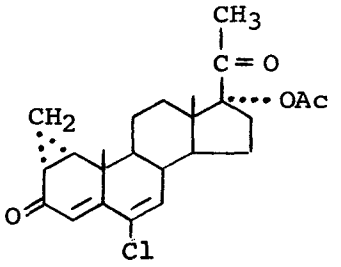


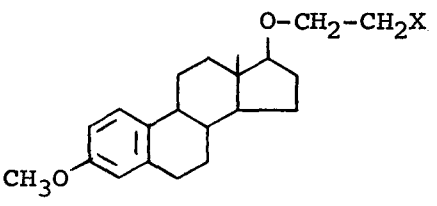
31

Estrogens

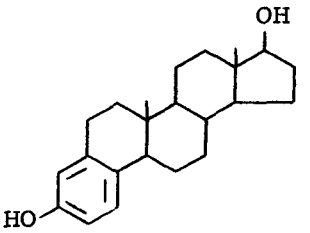
Recent reviews on estrogens and antiestrogens have been published.[48,58-60]

The 2-chloroethoxy ether 32 and the 2-fluoroethoxy ether 32a have shown[61] estrogenic potencies in the mouse uterine assay of 4 times estrone when administered orally but only 0.5 and 0.05 times estrone on subcutaneous administration. The pentacyclic

phenol 33 administered to the castrate female mouse exhibited[62] estrogenic activity of 1/4000 that of estrone. A number of C-19 functional steroids[63] also show weak estrogenic activity.

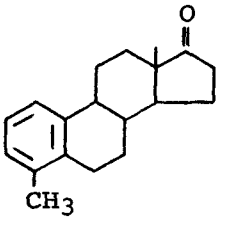


32 X = Cl
32a X = F



33

The lipodiactic-estrogenic ratio has been determined for a number of 3-desoxyestratrienes, and the 4-methyl compound 34 has a ratio of 150.[64] Several other compounds have been studied for their effect on blood lipids. These include the 16β,17β-pyrazoline 35,[65] the 2-methoxymethyl compound 36,[66] the 13β-ethyl compound 37,[67] the androstane-2-propionic acid 38[68] and several 1-methylequilenine compounds.[69] All of these compounds lower blood cholesterol.



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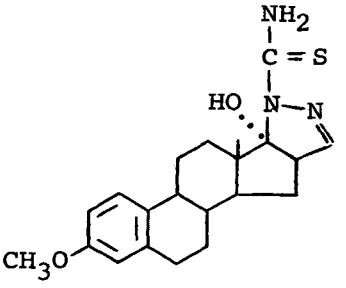

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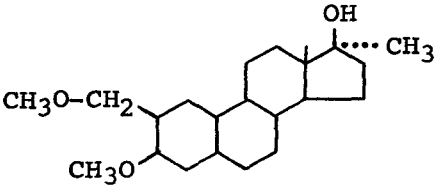

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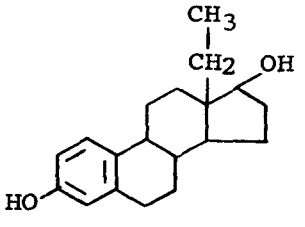

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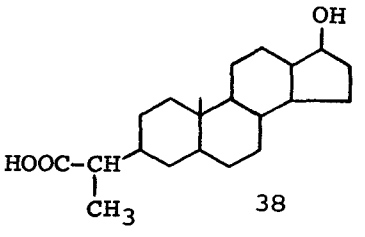

38

Corticoids, sex hormones and nonhormonal compounds are known to inhibit the biological activity of estrogens. A compound which shows both antiandrogenic and antiestrogenic properties is 26.[51]

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Chapter 20. Non-steroidal Antiinflammatory Agents

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Introduction. Non-steroidal antiinflammatory (AI) agents are of interest for the management of inflammation and pain in scores of rheumatic and other inflammatory conditions. Some may be found useful as antiasthmatic agents.[1] The last few years have seen a rapid development in this area in new clinically active drugs, in new and improved laboratory test methods, in proposals of mechanisms of action of AI agents, and in the number of laboratory publications and patents disclosing new agents. A recent review[2] covers background material. The best introduction to current thinking is found in the published Milan Symposium on Non-steroidal AI Drugs[3] and a chapter by Whitehouse[4] on biochemical and pharmacological properties of AI drugs. There is a need for improved antirheumatic agents. The status of currently available agents is indicated in the theme of a recent A.M.A. panel discussion on the subject[5]: "Risk vs. Benefits in Rheumatology," and in other reviews.[6,7]

Test Methods. Inflammation is a complex biological process.[4] Attempts to duplicate the clinical state in the laboratory have been largely empirical. The result is a variety of test methods,[3,4] and a complex assortment of compounds claimed to have AI activity. Order is gradually appearing. With new clinically active agents available in addition to phenylbutazone (indomethacin, flufenamic acid), Winter[3a] has reexamined various rat paw edema tests. He concludes that the widely used tests employing formalin, egg white and serotonin as phlogistic agents are unsuitable (insensitive). He also found that sterile kaolin does not produce edema. These and other irritants (e.g., yeast and mustard) are known to give positive results with antihistamines, antiserotonins and diverse other agents.[3a,c] The most reliable tests appear to be the UV erythema test (UV, guinea pig), the antibradykinin test (B, guinea pig bronchoconstriction), and the cotton pellet granuloma test in the rat (GC). A rat paw edema test using carrageenin[3a,10,11] (E_C) has been widely adopted. It is a considerable improvement over older E tests, although false "positive" results are obtained with high doses of a variety of medicinal agents.[10] One also finds frequent reference to the granuloma pouch assay (G) and a new and interesting test system, an adjuvant-induced arthritis in rats (A)[53,54]. The latter test, or ones like it, offer hope for finding drugs which will provide preventive or curative rather than symptomatic treatment of inflammatory diseases. Steroids are inactive in the UV and B tests, but active in the G, GC, E and A tests.

In the discussion which follows, a code system will be used to indicate approximate activities relative to phenylbutazone (PB), and the test method used. Thus 2 E_c indicates a compound is about twice as active as PB by the rat paw edema test using carrageenin. (Other abbreviations: y=yeast, d=dextran.) In addition to the above tests most AI agents are active in antinociceptive tests involving inflamed tissues[3h,13,14] and in various writhing rodent tests (presumptive of analgetic activity), often in proportion to their AI activity. They are also antipyretic. These agents are commonly referred to as antiinflammatory analgetics or antiphlogistic agents to distinguish them from strictly central analgetics. A number of non-steroidal antirheumatic agents appear to act by a different mechanism from the antiphlogistic agents. These include gold preparations,[6] antimalarials,[4] proteolytic enzymes,[3d] and agents specific for gout.[8] These will not be covered in this review.

Receptor Site. In 1964 the Parke-Davis group[15] proposed a hypothetical receptor to accommodate a number of known AI agents; Shen[3b] presented a similar receptor based on an analysis of the indomethacin structure and substitution effects. The main features, outlined in fig. 1, are a large flat area, a trough to accommodate an out-of-plane group (such as an aryl ring), and a cationic site to accommodate an acid anion (or unprotonated amine?). It will be seen that a number of agents described below can be fitted to this receptor. The correlation is probably higher among known UV-active compounds. These agents will be discussed by broad chemical classifications.

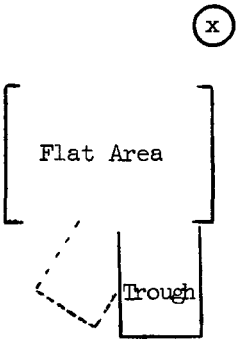
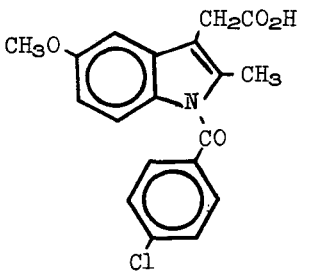


fig. 1



I

Arylacetic Acids. This class probably represents the area of greatest research effort in the AI field today. One of the most significant events in medicinal chemistry in 1965 was the NDA approval and marketing of indomethacin (I, Merck; 2 E_c, 85 GC, 3 UV, 4 B, 25 A; potency in man is several-fold PB). The chemistry,[3b] pharmacology,[3a,16] and clinical reviews[5-7] are available.

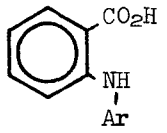
Clinical reports have appeared[17] indicating that the diethylaminoethanol salt of α-ethylbiphenylylacetic acid (namoxyrate, Warner-Lambert) is an effective AI agent and analgetic. Superiority of the salt over the free acid (mouse writhing test) is the basis of a patent claim.[18] Boots Pure Drug has received a patent on a series of biphenylyl- and α-methylbiphenylylacetic acids (substituted on Ar), but omitting α-methylbiphenylylacetic acid itself.[19] The latter, along with numerous carboxyl derivatives, is now covered by a Merck patent.[20] Patents on 4- and 5-phenyl-1-naphthaleneacetic acids, and Ar-substituted and α-alkylated derivatives have appeared.[21] All the above are claimed to be AI agents.

4-Isobutylphenylacetic acid (ibufenac, Boots Pure Drug; 0.2-0.4 UV) has been found to be clinically as effective as aspirin, at half the dose, in various rheumatic conditions, but hepatotoxicity may limit its usefulness.[22] An extensive Merck patent has now appeared covering hundreds of Ar-substituted 4-alkyl- and 4-cycloalkylphenylacetic acids as AI agents.[23]

The effect of α-substitution on the AI activity of various arylacetic acids was the subject of two recent publications.[24,25] Substituted 1-naphthaleneacetic acids were superior to the corresponding phenylacetic acids. The best compounds in the 1-naphthalene series (e.g. α-furfuryl, 0.25 UV) were not much more active than the unsubstituted acid (0.12 UV). Buu-Hoi[26] finds AI activity in p-n-butoxyphenylacethydroxamic acid (<1 E_c) but doesn't compare this with the corresponding carboxylic acid.[cf. 12]

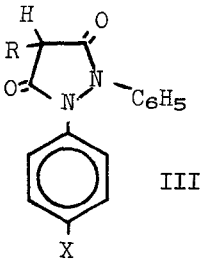
N-Arylanthranilic Acids. This series includes the most potent agent reported in the UV and antibradykinin tests: N-(2,6-dichloro-m-tolyl) anthranilic acid[27] (IIa, CI-583, Parke-Davis; 15 UV, 14 GC, 32 B), now under clinical trial. Flufenamic acid (IIb; 1.6 UV, 3.6 GC, 4 B, 4.5-9 E_c, >1 A) also under clinical trial, and mefenamic acid (IIc; 0.5 UV, 0.6 GC, 4 B, 2.6 E_c), being marketed in a number of areas as an antiinflammatory analgetic, are other members of the series. While the peripheral site of pain relief inferable from their UV activities[15] has been confirmed in rats for both mefenamic and flufenamic acids,[14] the additional existence of a "presumably central" action[15] has been questioned.[14] The analgetic activity of mefenamic acid in man continues to be confirmed.[e.g.56]

An anthranilic acid substituted with the antimalarial quinoline nucleus, 2,3-dihydroxypropyl N-(7-chloro-4-quinolyl)anthranilate[28] (glaphenine, 0.1 UV), has been introduced in France as an analgetic.[29] A series of 2-anilinonicotinic acid derivatives[9] is claimed to have analgetic and AI activity (E_d, $E_{bradykinin}$).



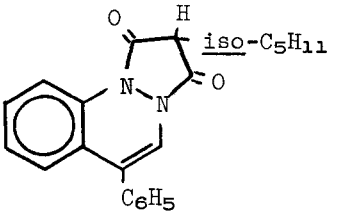
II

Ar
a) 2,6-Cl_2-3-$CH_3C_6H_2$
b) 3-$CF_3C_6H_4$
c) 2,3-$(CH_3)_2C_6H_3$

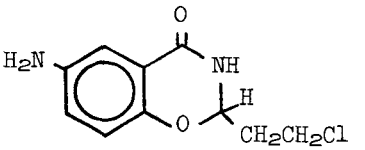


III

a) R=n-C_4H_9; X=H
b) R=n-C_4H_9; X=OH
c) R=  ; X=H



IV



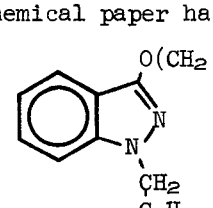
V

Pyrazolidinediones. Phenylbutazone (IIIa) was the first of the potent non-steroidal AI agents. Its AI activity was recognized in man by chance in 1952. Due to its toxic liability its use in the U.S. is generally limited to short periods. A metabolite, oxyphenbutazone or metabolite I (IIIb), is commercially available and reportedly less toxic than PB. Much synthetic effort has been expended in this area without notable success to date. A recent patent[30] claims IIIc to be active in man as an AI and analgetic. An interesting variation for its potential fit to the hypothetical receptor is found in IV[31] (1+ E_{kaolin}). 4-t-Butyl-2-phenylisoxazolidine-3,5-dione[32], one of a series, is reported to be about equal to PB in inhibiting a gnawing response in guinea pigs induced by intraperitoneal talc.

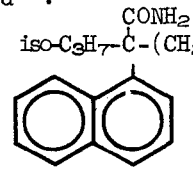
Salicylic Acids. With this class we come to one of the oldest antirheumatic agents and the first drug of choice in this field— aspirin. While pharmacologists and others had felt it to be so for some time, the first conclusive clinical evidence that aspirin is an antiinflammatory agent appeared in 1965.[33] Aspirin (0.1 UV, 2 B, 0.5 E_c, 0.2 A) is more active than salicylic acid in most pharmacological tests for antiinflammatory and analgetic activity,[e.g. 3e] and is probably active as such in vivo. Salicylamide does not have AI activity. A cyclic derivative of the latter, benz-1,3-oxazine-2,4-dione (carsalam), apparently available in Europe[34], is claimed to be an analgetic and AI agent. Another clinically active cyclic derivative is 2-(β-chloroethyl)-2,3-dihydro-4-oxo-6-aminobenz-1,3-oxazine[3f,35] (V,A350; 1 E_c). In laboratory tests the latter is more active than the desamino compound known as chlorthenoxazine.

Alkylamines. There is a rapidly growing list of amines for which AI activity is claimed. Some have rather close counterparts in acidic series discussed above and also would appear to complement the receptor area outlined. It will be of special interest from a pharmacological standpoint to follow the progress of these agents through clinical trials since many of these are inactive in the UV test.

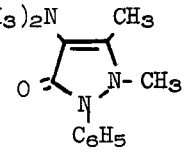
Benzydamine, 1-benzyl-3-(γ-dimethylaminopropoxy)-1H-indazole hydrochloride (VI, Angelinini Francesco; < 1 E_C, 1 G, 0 UV), is reported to be a clinically active antiinflammatory analgetic[3g]. Silvestrini[3h,36] has discussed his approach to screening and the interpretation of test results. In a publication on the pharmacology[36] the authors state: "As viewed from a quantitative angle [benzydamine] seems to be—according to our own experience—the most powerful analgesic-antiinflammatory drug so far described". (Clinically? This statement is difficult to understand from the data given; apparently the emphasis is on analgesic.) A chemical paper has appeared[37].



VI



VII



VIII

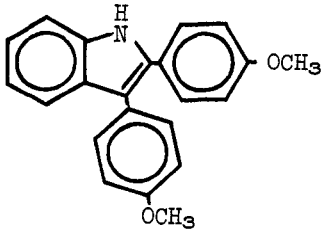
A series of α-isopropyl-α-(β-dimethylaminoethyl)naphthylacetamide (VII, naphthypramide; 1 E_C) and related derivatives has appeared out of the Instituto De Angeli[38]. As with the arylacetic acid series of Durant et al.,[24] the naphthyl derivatives were superior to the phenyl analogs. More recently 4-isobutylnaphthypramide (analogous to ibufenac) has been reported to be distinctly superior to naphthypramide[39]. N,N,1-Trimethyl-α,α-diphenyl-3-pyrrolidine acetamide (AHR-840; 0 UV) is reported to be comparable to PB in analgetic tests on the inflamed rat paw and to inhibit alloxan-induced writhing and granuloma formation.[40]

1-[3-Ethoxy-3-(p-chlorophenyl)propyl]-4-phenylpiperazine (2-3 G and GC) and several related compounds are reported to have antiinflammatory activity.[41] This property could be separated from antihypertensive and adrenolytic activity found in other members of the series.

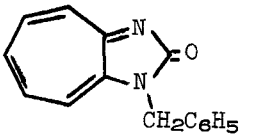
Other Amines. The compounds in this group differ from those just preceding in that an amine function is attached directly to a heterocyclic ring. The oldest AI compound of this type is aminopyrine, (VIII; 0.14 UV) considered too toxic by the U.S. FDA[55] for general use. 5-Amino-1-phenyltetrazole (fenamol, Armour) is reported to be active clinically in rheumatic conditions.[42] No pharmacological paper has appeared. A series of 3-amino-2,1-benzisothiazoles was reported to have antiinflam-

matory as well as gastric antisecretory activity[43] (e.g. 3-ethylamino-4 B; 6-chloro-3-dimethylamino- 1 UV, 0.5 B). There was no correlation between AI and antisecretory activities. A patent on a series of 3-aminoalkylamino-1,2-benzisothiazoles with AI activity has appeared[44] (E_d, $E_{croton\ oil}$).

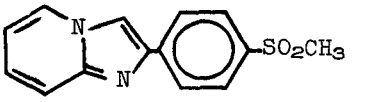
Other Agents. Two compounds under clinical trial are of considerable interest since they differ from known active non-steroidal agents in not containing "functional" substitution. These are 2,3-bis(p-methoxyphenyl)indole[45] (IX, indoxole, Upjohn; 1-5 E_c, 1 A) and 1-benzylcycloheptimidazol-2(1H)-one[46] (X, RCH-314, Sankyo; > 1 E_d). The latter compound is a feeble uncoupler of oxidative phosphorylation (in vitro), leading Whitehouse[47] to predict poor AI activity. Another "nonfunctional" series has been reported[48] which included 2-(p-methylsulfonylphenyl)imidazo[1,2-a]pyridine (XI) with activity, at comparable 1/4 LD_{50}'s, greater than PB in E_c, E_y and various antinociceptive tests. Several 3-amino substituted derivatives were active and a 3-acetic acid series (analogous to indomethacin, etc.) is promised.



IX



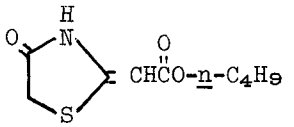
X



XI

Dimethylsulfoxide has been said to be beneficial in the relief of rheumatic pain (reviewed[49]). It has recently been withdrawn from most clinical trials because of reported eye damage. In the view of the U.S. Food and Drug Administration[50] (March 1966), there is a lack of substantial evidence of the effectiveness of DMSO.

Injection of Freund's adjuvant into the hind foot of a rat causes an inflamed lesion in the injected foot, followed after about ten days by inflamed secondary lesions (arthritis) in the other feet.[53] Phenylbutazone inhibits the primary lesions and, to a lesser extent, the secondary lesions. An interesting development[53] is the finding of a compound which prevents the appearance of secondary lesions, while not affecting the primary lesions. The compound is 2-butoxycarbonylmethylene-4-oxothiazolidine (XII; slight E_c, 0 E_{kaolin}). Only clinical trial will determine if these results imply a new kind—or any kind—of antirheumatic activity.



XII

Mechanism of Action. Whitehouse[3i,4] rather persuasively presents arguments to support the proposal that an important property of non-steroidal antiinflammatory agents is their ability to uncouple oxidative phosphorylation. The correlation between these activities is high, especially considering that uncoupling activity is determined in vitro. Another in vitro test has been devised[51] based on the high affinity of AI agents for lysyl ε-amino groups of plasma albumin. Biochemical implications, including a possible connection with the uncoupling of oxidative phosphorylation, have been discussed.[52]

The role plasma kinins may play in inflammation has been reviewed by Lewis.[3j] A mechanism of action of AI proteolytic enzymes in terms of the destruction of bradykinin has been considered.[3d] It has been found[11] that inflammation brought on by carrageenin in the rat paw edema test differs from that caused by some other irritants and may be due to the release of bradykinin.

While the last few years have seen considerable progress in the non-steroidal antiinflammatory field, this seems to be only the beginning of a period of even greater activity and progress as developments in the biochemical, pharmacological and medicinal chemical areas are translated to developments in clinical areas.

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Chapter 21. Agents Affecting Blood Enzymes

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This review is concerned with agents which act by alteration of enzymes in the circulating plasma, and will not deal with compounds which influence blood enzymes coincidental to other pharmacologic effects. The drugs to be reviewed are primarily those which alter clot formation or dissolution, or hemorrhage as it relates to clotting factors.

In the area of directly acting (heparin-like) anticoagulants, there are new semisynthetic heparinoids[3,31,32] polynucleotides with phosphate groups rather than sulfates as in heparin[4,34,35] and natural agents which are chemically quite different, but exhibit direct anticoagulant action not neutralized by protamine.[36] These studies confirm that heparin-like effect is not a sole function of the negatively charged groups. A review of positively charged compounds, particularly metal complexes[5] indicate that heparin neutralizing potency is not a simple result of positive charge.

There is interest in the antithrombotic activity of sulfate-free low molecular weight dextrans which do not significantly prolong test tube clotting time.[7] The unexplained hemorrhagic complications of the use of dextran as a plasma expander may have a common origin with this alleged antithrombotic activity.

So far the newer heparinoids show little if any promise of significant advantage over heparin itself. None has yet reached the U. S. market. Oral efficacy has not been achieved with practical dosage of heparin or its analogs either as anticoagulants or as lipoprotein lipase activators.

A paradoxical use of heparin to prevent or control hemorrhage has been described[6] in patients with the so-called "depletion syndrome," a condition in which clotting activators released into the blood as a result of disease, surgery, or parturition, cause excessive consumption of clotting factors, including fibrinogen, which in turn leads to hemorrhage. Heparin, by preventing the hypercoagulability which initiates these events, may also prevent or control the resulting hemorrhage. Accurate assessment of the clotting pattern is critical to this newly proposed use for heparin.

Among the hypoprothrombinemic agents (indirect anticoagulants), several new active indandione and coumarin derivatives have been described[8,10,33] including some from natural sources, some with polymeric structures[9] and some which are presumed to be of primary interest as coronary dilators.[11]

Additional information concerning hypoprothrombinemic agents include: further observations of the relationship of vitamin K and anti-vitamin K activity to structure;[10] the influence of other drugs, including steroids[13] on the distribution and metabolic fate of coumarins;[1,14] indandione toxicity of the kind not seen with coumarins.[2]

The concept that the initiating event of clinical thrombosis is the clumping and breakdown of platelets has resulted in the study of agents which are not anticoagulants in the sense of prolonging clotting time, but which decrease excessive stickiness or adhesiveness of platelets.[15] A diverse group of compounds which have little in common structurally or pharmacologically have been reported to reduce platelet stickiness, i.e., the hypolipidemic agent Atromid,[16] the coronary dilator Persantin,[17] and the uricosuric agent Anturane.[18]

In the search for clot dissolving agents, the trend has been away from fibrinolysin itself and toward activators of naturally occurring precursors of fibrinolysis. Difficulties with streptokinase antigenicity has caused more attention to be paid to non-antigenic urokinase[19] in spite of considerable production problems related to the need for large volumes of human urine as the starting material. Its full therapeutic value is yet to be proved empirically in the clinic. This need for empirical proof of efficacy is at least as great for a group of non-enzymatic compounds which activate fibrinolytic proenzymes. Some of these[12,20] appear to be outgrowths of the observation that parenteral nicotinic acid induces an intense but short-lived fibrinolytic response in man,[21] while others[22] probably can trace their origin indirectly from the well known *in vitro* fibrinolytic action of concentrated solutions of urea.

The inhibition of excessive fibrinolytic activity as a means to control hemorrhage has resulted in the marketing of E-aminocaproic acid[23] and the development of active analogs.[24,25] Large doses of EACA are required in spite of its high potency and good absorption because of its very rapid urinary excretion.

Another approach to the control of bleeding is based on the presumed activation of Hageman Factor (Factor XII) by ellagic acid,[27,28] even though patients with marked Hageman deficiency do not suffer from bleeding problems. The mechanism of the alleged influence of various steroids on thrombotic[29] and hemorrhagic[30] tendencies remains unclear.

This reviewer has previously expressed concern about the absorption, and, therefore, efficacy of orally administered enzyme preparations whose action is presumed to be a result of their entry into the blood in active form.[26] He is not aware of any new data to alter this position and has yet to be satisfied that any of the pharmacologic actions of oral enzymes are due to the presence of their specific enzymic properties in blood or tissue after oral administration.

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Section V - Topics in Biology
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Chapter 22. Molecular Aspects of Drug-Receptor Interactions

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> "...it has always been clear that no drug action can be understood completely until it is pursued to the level of molecular interactions, and now such pursuits can increasingly become realistic goals of research..." - Avram Goldstein[1]

In the absence of any guiding or constraining precedents, this first of an annual series of brief reviews on molecular aspects of drug-receptor interactions will emphasize several newer techniques and concepts of broad applicability that appear destined to play an increasing role in uncovering knowledge about the molecular nature of drug-receptor interactions. The first three sections discuss such topics briefly and provide leading references. The fourth section reviews recent developments in receptor theory of a more specific nature. Space considerations have limited its scope to publications dealing with the autonomic and central nervous systems. Since the present subject is not well suited to review on a calendar basis, coverage has been expanded to include other important publications of recent vintage.

The formidable problem of seeking to understand the action of a drug in molecular terms can only be faced after certain more fundamental questions are answered. Where does the drug act morphologically? How does it act functionally? Which of its actions directly involve the receptor site of interest? Finding the answer to these questions can often prove to be a major problem. Nevertheless, the present review largely ignores these preliminary considerations to emphasize the ultimate analysis -- the interpretation in molecular terms of the interaction between drug and receptor. It has become increasingly feasible to consider drug action at this level in recent years because of the progress made toward understanding nucleic acid biology, protein ternary and quaternary structure, the nature of enzymes, and the mechanism by which the activity of the latter is controlled. The section to follow discusses the importance of this new knowledge as a source of direct insight regarding the nature of receptors.

Sources of Insight Regarding the Nature of Receptors. In seeking to understand the nature of drug-receptor interactions in molecular terms, the greatest obstacles usually relate to the macromolecular partner -- the receptor. The problem is most often approached by indirection, by assuming "that small molecules whose biological action is specific and structure-dependent display a high degree of molecular complementarity toward the site at which they act."[2] Although appreciable progress has been made in this way and a good deal more can be anticipated, it is well known that the

synthesis and meaningful biological evaluation of large numbers of analogous molecules can be an exceedingly laborious exercise that often proves relatively unrewarding.

As we come to appreciate more fully the subtlety and dynamism of enzyme-small molecule interactions (assuming that we accept the inevitability that directly or indirectly drugs act upon enzymes) it becomes increasingly apparent why this classical approach is so often limited in effectiveness. In the eloquent words of a Lancet editorial[3] entitled The Shape of Molecules to Come: "...the so-called active site of an enzyme is not a fixed circumscribed area endowed with catalytic properties but a moving constellation of atoms, a gesture rather than a limb...." The realization that significant conformational changes can and probably do occur in receptor systems as the result of their binding drugs as substrates, inhibitors, allosteric effectors, or otherwise, suggests one very good reason why it is so difficult to conceive their nature merely by assuming molecular complementarity with interacting drugs.

More direct means of gaining insight are clearly needed. Perhaps the best such means presently available involves the extrapolation of newer knowledge and concepts regarding the structure of proteins[4,5], the regulation of enzyme activity[6], and the nature of feedback control mechanisms at the molecular level[7]; for example, the views of Koshland[8,9] on enzyme flexibility and induced fit, and those of Monod and his colleagues[10-12] on allosteric proteins and transitions, and on the molecular interactions controlling cellular metabolism.

The kind of structural information currently being garnered by various instrumental techniques may also be expected to provide a powerful stimulus to thought about the nature of receptors. A case in point are the Nobel Prize-winning, X-ray crystallographic studies on the structure of hemoglobin[13-18]. Not that one expects to isolate, crystallize and analyze the diffraction patterns of very many receptors in the next little while! But in the sense of gaining insight regarding fundamental principles, one must recognize the potential value of a method that can detect and define remote conformational changes in a macromolecular structure that arise from its interaction with a small molecule. Separate X-ray analyses of reduced hemoglobin and oxyhemoglobin have afforded evidence of a 7Å increase in distance between the heme groups of the two β-chains, which occurs upon oxygenation! These dramatic findings should serve to temper any reservations about the limited value of structural information obtainable from proteins in the crystalline state.

The fascinating hemoglobin story was well reviewed during 1965 in the above-mentioned Lancet editorial[3], by Perutz[19], and by Muirhead[20]. Other X-ray structure analyses that reveal the potentialities of the technique for studying complexes of proteins with small molecules deal with hen egg-white lysozyme-inhibitor complexes[21,22], the binding of azide, cupric and zinc ions to sperm whale myoglobins[23,24], and α-chymotrypsin inhibited with diisopropyl fluorophosphate and with a number of sulfonyl fluoride inhibitors[25].

Despite the great technical complexities of X-ray crystallographic analysis, the swift progress being made in the automation[26] of both instrumentation and methods for handling the data produced, serves to engender hope that significant contributions to our understanding of the nature of receptors will eventually be forthcoming from this quarter, earlier pessimistic comments[27] notwithstanding.

Space limitations preclude a review of other newer instrumental methods significant to the problem of characterizing drug-receptor interactions. Briefly, optical rotatory dispersion and circular dichroism measurements[28] deserve mention as a very effective means of detecting changes in protein ternary structure. A recent study[29] of conformational changes induced in chymotrypsin by various substrates and inhibitors is a case in point.

The application of nuclear magnetic resonance spectroscopy (NMR) to biological problems has been well described by the pioneering Cohn[30] and Jardetsky[31,32] groups. The method may be of particular value in studying the nature of drug-receptor binding (e.g., a recent analysis[33] of thiamine-indole complexes). A sophisticated example of the use of both NMR and electron spin resonance spectroscopy to define the structure of enzyme-substrate-activator complexes is provided by recent studies[34,35] on pyruvate kinase, which take advantage of the paramagnetic properties of manganous ion to elucidate the reaction pathway and the structure of the ternary intermediates.

Although not strictly an instrumental technique, the use of deuterium isotope effects to investigate the nature of bioreceptor-substrate complexes warrants mention here because of its rather general applicability. A recent review[36] by Belleau, who has led in the development of this approach, summarizes its present status. Studies are described that relate to receptor- and enzyme-bound acetylcholine, to the histamine-histaminase system, to the nature of monoamine oxidase and dehydrogenases more generally (determination of whether they are flavin-dependent or pyridine nucleotide-dependent), and to "morphine N-demethylase" and the morphine receptor.

Quantum Mechanical Approaches to Determining Localized Electron Densities - Until such time as a macromolecular receptor system can be directly characterized with facility, it will remain necessary to understand as much as possible about the underlying nature of the small molecules that interact with it most strongly and specifically. One challenging aspect of that analysis involves the determination of localized electronic densities that may play a significant role in the receptor interaction. The use of molecular orbital calculations toward that end has been urged repeatedly by the Pullmans[37], and Szent-Györgyi[38], and provocative analyses have been presented for important series of electron-donor molecules of pharmaceutical interest such as the purine bases, phenothiazines, indoles and the like. The advent of high-speed computers has greatly facilitated the analysis of complex organic molecules, and this will doubtless stimulate further interest in applications to drug-related problems.

In assessing the potentialities of the approach, it is necessary to recognize that the use of molecular orbital calculations has on occasion spawned some unlikely conclusions regarding the nature of drug-receptor interactions. Although it is probably correct to attribute some of these to the inherent complexity and limitations of quantum mechanical calculation methods, a more common failing appears to be lack of recognition that a multiplicity of factors often underlie the observed biological activity. Recent studies by the Hansch[39,40] and Purcell[41] groups demonstrate a means of avoiding this pitfall. Their findings from molecular orbital calculations are placed into perspective by weighing them alongside other, often unrelated molecular characteristics such as partition coefficients, as a basis for drawing conclusions.

Quantum biochemistry has been discussed in several books[37,38,42,43], one of the most stimulating being by Szent-Györgyi[38]. A recent article on quantum chemistry in drug design[44] offers a convenient introduction to the subject. Texts on calculation methods are now available in profusion. Intriguing analyses based on molecular orbital calculations have been made of charge-transfer complexes of indoles[45], cholinesterase inhibitors[46], and hallucinogenic indoles and methoxylated phenethylamines[47].

The related subject of charge-transfer complex formation has been reviewed by Kosower[48], who discusses biological systems. Analyses of such complexes[49], especially those involving flavin nucleotides[50,51], have been described.

Mathematical Approaches to the Correlation and Analysis of Structure-Activity Data - Attempts to systematically correlate the physicochemical properties of molecules with their biological activities have been recorded since the turn of the century. Needless to say, many earlier efforts involved only the simplest forms of data manipulation and analysis. Inevitably, what little knowledge about drug receptors they produced was hard won. From about 1951 on, interest has focused increasingly on the possibility of utilizing linear free energy relationships[52] as expressed in the Hammett equation to interrelate the physicochemical and biological contributions made by a structural moiety to the parent molecule. As the result of efforts by Hansch and his colleagues[39,40,53-64], this mathematical approach has gained steadily in sophistication, and with the advent of "simple-language" computer programs, in effectiveness as well. Of particular pertinence here, recently published regression analyses have demonstrated that this approach can offer insight into the nature of drug-receptor interactions. Contributions to knowledge from this source have been of two kinds, those arising by the disentanglement from intrinsic activity of drug dynamic factors -- the ability of the biologically-active molecule to reach its site of action effectively, and those provided by identification of the specific hydrophobic bonding, electronic, and to a lesser degree steric factors that influence intrinsic activity.

It makes particularly interesting reading to trace the advent of the Hansch equation in chronological fashion, against a backdrop of earlier attempts[65-69] to treat biological data mathematically. In 1962 Hansch[55] suggested that lack of success experienced in applying Hammett linear

free energy relationships to rationalize the effect of aromatic substituents on plant auxin activity was the result of ignoring differences in the rate of penetration of various congeners to their site of action. He thereupon introduced the comparative free energy-related substituent constant, π, to reflect that parameter, leading to the modified Hammett equation which now bears his name in vernacular usage:

$$\log \frac{1}{C} = -K\pi 2 + K'_{\pi} + \rho\sigma + K''$$

where C is the molar concentration of drug producing a specified biological response, K, K' and K'' are constants for a given series of congeneric drugs, and ρ and σ retain their familiar significance.

From its humble beginnings in the plant kingdom, the use of this mathematical expression came into full flower in 1965, with six important publications[40,59-63] emanating during that period from the computer of its originator. The impressive results obtained in these studies should serve to dispel any lingering doubts as to the breadth of applicability and incisiveness of this method.

The π term is commonly defined as the difference between the logarithm of the partition coefficient (usually 1-octanol/water) of the parent molecule and that of a derivative bearing the substituent in question. On occasion it has proven possible to use parachors or chromatographically-determined substituent constants in place of partition coefficients as the basis for the π term. Other electronic parameters have been successfully employed in place of the Hammett σ constant when more pertinent. These include pK_a values and electron densities obtained by molecular orbital calculations. Interestingly enough, relative to the discussion in the previous section, it was lack of success in attempting to develop a quantitative relationship between the biological activating ability of functional groups and their relative electronegativity as determined by molecular orbital calculations that first led Hansch to appreciate the need for a term reflecting the different penetration characteristics of various congeners.

The Hansch approach is not without shortcomings. By and large it cannot cope too successfully with steric factors, or with metabolic inactivation processes, except in certain favorable instances. Difficulties can be encountered in predicting π from partition coefficients, particularly where substituents that can ionize at physiological pH are involved. Despite these limitations, it seems safe to predict that as computers become commonplace, along with programs for treating data of this type with facility, use of the Hansch equation (or possibly extensions of it) will increase appreciably.

The power of the method is well-exemplified by a recent analysis[64] of structure-activity relationships among phenylcarbamates, anilides and phenylureas inhibiting photosynthesis, which concludes that the primary effect on biological activity of aromatic ring substituents in such structures arises from the hydrophobic bonding power of the substituents. This conclusion was in disagreement with a previous paper[70] that stated, on the basis of molecular orbital calculations, that a high electron density in the ortho position of the aromatic ring was the factor responsible for

biological activity. Interestingly enough, the latter paper has subsequently been withdrawn[71], due to a fundamental error in the molecular orbital calculations employed, which invalidated its conclusions. The two papers taken together constitute an interesting case study of contrasting methods of analyzing structure-activity relationships.

A different approach to the mathematical treatment of structure-activity data, based on the assumption that there is some additivity of substituent contributions in analogous structures has been described by Free and Wilson[72]. The method has been applied recently to an analysis of the cholinesterase inhibitory properties of a large series of N-alkyl substituted 3-carbamoylpiperidines by Purcell[73]. In its present state of development, it does not seem to offer the same potentialities for gathering insight into the molecular nature of drug-receptor interactions as does the Hansch equation.

Studies on Autonomic and Central Nervous System Receptors.

Analgesics - Portoghese[74-76] has reinterpreted existing structure-activity data in terms of differing modes of analgesic-receptor interactions. He has suggested that the mode of binding of two different series of compounds can be recognized as similar or different, by determining whether comparable activity changes occur when substituents on the basic nitrogen atom are varied in identical fashion. A linear free-energy relationship can be demonstrated to exist when binding modes are similar. This method, which obviously has broad implications, has recently been applied to an analysis of the mode of binding of cholinesterase inhibitors[41]. Earlier views on analgesic receptors are summarized in several recent reviews[77-80].

General Anesthetics[81] - During 1965, the compelling hydrate microcrystal theory of anesthesia put forth by Pauling[82], and its "iceberg effect" counterpart offered concurrently in 1961 by Miller[83], have been challenged in a paper by Paton and his colleagues[84], which asserts that the critical phase for general anesthetic action is a non-aqueous one. At a conference on Forms of Water in Biological Systems, however, the hydrate microcrystal theory was favored and new evidence cited in support of it[85].

Local Anesthetics - Recent studies[86-90] suggest that local anesthetics compete with calcium ion for binding sites on specific phospholipid molecules which are probably associated with the plasma membrane cation transport system of nerve cells. The nature of the phospholipid binding site involved has been suggested to be a phosphodiester on the basis of *in vitro* interaction studies with model compounds[90]. Structure-activity correlations among local anesthetic families have been made on the basis of determination of carbonyl bond order by infrared spectroscopy[91], regression analysis of structure-activity data (the Hansch approach)[39], and molecular orbital calculations[92]. In the latter case, the analysis was interpreted to favor charge-transfer complexing with thiamine as the basis for local anesthetic action. Compelling criticism of this viewpoint is offered in an excellent review[93] of the entire subject of the mode of action of local anesthetics.

Phenothiazine Central Depressants - Chlorpromazine and its analogs have been favored subjects for mechanism speculations since their advent as tranquilizers. A number of useful studies have been published which attempt to define their mode of action at the level of cell and organelle membranes and individual enzyme systems. Molecular orbital calculations[94,95], crystal photoelectric threshold studies[96], optical absorption and NMR spectra measurements[97,98] have all confirmed that the phenothiazines have significant electron-donor properties, which Szent-Györgi[38] has suggested to be the fundamental basis for their biological activity. Another important clue is the observation[99] based on surface tension measurements, that chlorpromazine complexes with ATP[100]. Coupled with the finding that the central nervous system depressant potency of analogous phenothiazine structures correlates with their ability to inhibit rat brain Na^+, K^+-activated, Mg^{++}-requiring ATPase[101], this raises the possibility that enzyme-bound ATP may constitute a receptor site for such drugs. Strong evidence to suggest complexing to other nucleotides in vivo also exists[102,103]. An interaction between flavin-adenine nucleotide-requiring enzymes and phenothiazine derivatives, has been noted in several studies. A recent one[104] establishes a correlation between effectiveness in inhibiting such an enzyme (D-amino acid oxidase) and central depressant potency. The general hypothesis[105,106] that the tranquilizing actions of phenothiazine derivatives relate to inhibition of both energy-producing reactions (such as oxidative phosphorylation at a level where FAD is involved) and energy-utilizing reactions (such as ATP hydrolysis supporting membrane transport processes) seems worthy of retention.

Autonomic Receptors - A number of papers from a symposium on the Interaction of Drugs with Receptors held in April, 1965, at the School of Pharmacy, Chelsea College of Science and Technology, London, have been published in Advances in Drug Research[107]. Of these, one that clearly reveals the impact of modern mechanistic biochemical thinking is the generalized conformational perturbation theory of drug action offered by Belleau[108,109]. This view, which has been influenced primarily by knowledge about cholinergic receptor phenomena, suggests that drug molecules regulate the catalytic activity of their particular target enzyme system by inducing a specific ordering (agonism) or non-specific disordering (antagonism) conformational change in its protein-binding surface. Because it goes beyond the earlier occupancy and rate theories of drug action in offering a plausible physicochemical basis for receptor phenomena at the molecular level, this theory is deserving of the most thoughtful attention. The accompanying critique of other so-called theories of drug action emphasizes the need to recognize the elementary molecular processes involved, and in this regard offers useful perspective on the overall problem. Several other papers[110-116] dealing with cholinergic agents are worthy of note, particularly those regarding muscarinic receptors.

At the Chelsea meeting, Bloom and Goldman offered a detailed molecular visualization of the nature of catecholamine-adenine mononucleotide interactions in adrenergic mechanisms[117]. In this so-called dynamic receptor hypothesis, the catecholamines are postulated to function by enhancing the rate at which specific phosphorolytic enzymes convert ATP to ADP (α-response) or cyclic 3',5'-AMP (β-response). The adrenergic receptors

are visualized as enzyme-substrate complexes (in contrast to the classical concept: receptor = enzyme), with the catecholamine hormones functioning in the manner of activator substances. The catecholamines are seen to interact directly with the bound nucleotide substrate, thereby enhancing its reactivity rather than that of the enzyme to which it is bound. This differs in concept from the above-mentioned conformational perturbation theory of cholinergic agonism, although the two views are not incompatible. Belleau has recently discussed the dynamic receptor hypothesis and offered certain alternative views, especially regarding the role of the catechol moiety at β-receptors, in a paper[118] that is one of more than seventy-five from the Second Catecholamine Symposium published as an 800-page volume of Pharmacological Reviews.

Pratesi and Grana[119] have summarized their extensive studies of structure-activity relationships among adrenergic substances. Triggle and his colleagues[120-123] have contributed new adrenergic receptor studies involving irreversible inhibitors of the β-haloalkylamine type, as has Chapman[124]. Iverson[125] has made the interpretation of pharmacological experiments involving adrenergic receptors a bit easier with his systematic studies of structure-activity relationships among substances inhibiting catecholamine uptake mechanisms. Noteworthy attempts have been made to characterize adrenergic α-receptors through the use of radiolabelled blocking agents of the alkylating β-haloalkylamine type[126,127].

Woolley and his colleagues have suggested that a specific substance with the properties of a ganglioside is the serotonin receptor in smooth muscle and brain[128-132]. Their compelling arguments are based primarily on evidence that serotonin-susceptible tissue may be rendered selectively insensitive by treatment with neuraminidase and EDTA, and that this process may be reversed by treatment of the desensitized tissue with a purified sample of appropriate gangliosides. It would seem in order to suggest that at this stage of knowledge the ganglioside in question might better be called a vital component of the serotonin receptor system, rather than the serotonin receptor.

Several recently published articles[133] deal with drug-receptor interactions more generally. Gill[134] emphasizes an analysis of the physical forces which determine the size and shape of drug and receptor molecules. Burger and Parulkar[135] point out various implications of bioreceptor theory to drug design. The review by Gourley[136] on basic mechanisms of drug action includes useful sections on cell surfaces and membranes. Watkins[137] has offered an explanation of the action of acetylcholine, γ-aminobutyric acid and glutamic acid on cell membrane permeability in terms of the structural and charge distribution similarities between these agents and the "polar head groups" of lecithin, phosphatidylethanolamine, and phosphatidylserine. Burgen[138] and Mackay[139] have each published generalized mathematical treatments of the drug-receptor interaction problem. Collier[140] has dealt with receptor induction in a general theory on the genesis of drug dependence.

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Chapter 23. Fate and Distribution of Drugs

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Inspection of literature citations indexed in 1964-65 in the Chemical Abstracts and Derwent Pooled Pharmaceutical Literature Documentation (Ringdoc) revealed over 800 references dealing with some aspect of the metabolism of drugs or chemicals, exclusive of normal biochemical constituents of the mammalian organism. The parsimonious page restrictions imposed by the editors and the limited ambition of the authors did not allow a complete coverage of this great number of publications. We have, therefore, confined our examination to pertinent papers appearing in the following 20 journals: Arch.Int. Pharmacodyn.; Arch.Biochem.Biophys.; Biochem.J.; Biochem.Pharmacol.; Curr. Therap.Research; Endocrinology; Fed.Proc.; J.Biol.Chem; J.Clin.Invest.; J.Med. Chem.; J.Pharmacol.Exptl.Therap.; J.Pharm.Pharmacol.; J.Pharm.Sci.; Life Sci.; Metabolism; Nature; Proc.Soc.Exp.Biol.Med.; Science; The Pharmacologist; and; Toxicol.Appl.Pharmacol. In addition, Ringdoc abstracts (since 1964) were examined and selected references are also included. It is estimated that the selected journals together with the additional references cited in the text contain over 90% of the published work on the biotransformations of drugs. To those few authors whose papers we may have missed, we offer our unprejudiced apologies.

Excellent reviews on drug metabolism have appeared since 1961 in every volume of the Annual Reviews of Pharmacology, except 1966. This major source of information has been supplemented by the recent review by Shuster[1] and, by a stimulating general discussion on the fate of drugs and therapeutic implications in a recent chapter by Brodie[2]. The appreciation of how the understanding of the metabolic fate of a drug can be of importance in its proper usage and in the interpretation of its toxicity has been well stated by Burns[3] and colleagues[4]; an outstanding example is also found in the work by the Millers[5] on a select group of chemicals and their carcinogenicity. The proceedings from special pharmacologically oriented symposia held in Prague[6] in 1963 and in New York[7] and Chicago[8] in 1964 have been printed and contain a wealth of information pertinent to drug metabolism. The specialized field of the metabolism of natural and synthetic steroids is covered in the revised encyclopaedic compilation of Dorfman and Ungar[9] and needs no further reference.

With this concentration of knowledge so readily available, it appeared that the readers interest would best be served by bringing the available information up to date. Considering the chemical background of the likely reader of the Annual Reports in Medicinal Chemistry, we have orientated the review to the actual biotransformations that have been reported in 1964-65. Thus, we have not reviewed the voluminous and illuminating reports on the nature of the enzymes that metabolize drugs[10]; the mechanism by which enzymes catalyze these transformations[11]; on the dependence of enzymic activity on species[12,13], strain[14-16], age[17,18], sex[19-21], on the induction of increased quantities of these enzyme systems by chemicals and drugs[22], and on the relationship of "drug metabolizing enzymes" and the metabolism of steroidal hormones[23-25]. We have selected the above 1964-65 references to enable the reader to make convenient acquaintance with these specialized areas.

The review is written for a reader acquainted with approaches used by the medicinal chemist. Data on compounds, whose metabolism is reviewed, are

arranged in tabular form and include:main pharmacodynamic property;chemical name, or, if available, generic name as listed in the Merck Index; structural formula(an asterisk above the name indicates study done with radioactive drug) with arrows indicating sites of metabolic transformation; these are also described in the accompanying text. Abbreviations used are: UCC, unchanged (parent) compound; (M),major; (m),minor; (tr),trace; (A),biologically active; conj., conjugation; gluc., glucuronide; sulf. or SO_4, sulfate. Whenever deemed desirable, a comment was added. The compounds are arranged arbitrarily according to increasing structural complexity.

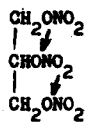
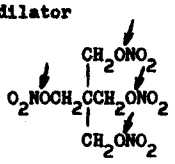
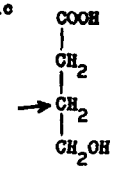
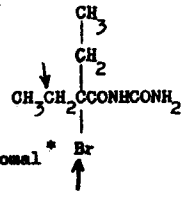
Inspection of the chemical fate of the 90 diversified structures indicates that the compounds underwent relatively few types of biotransformations. This serves to illustrate the concept of minimal substrate specificity. It is generally accepted that foreign compounds, including drugs, are transformed by a specialized group of enzymes to products that are less lipophilic and thus more readily excreted from the body. The concept still appears to be true that lipid solubility facilitates the metabolism of a foreign organic compound. However, in recent studies, lipid solubility had little effect on the demethylation of a series of similar compounds in vitro[117],or on the metabolism of sulfonated arylazanaphtol dyes in vivo[118]. Hence, other unknown factors may operate to control the rate of metabolism.

Most of the chemical transformations shown in the tables might have been anticipated, but the reduction of a sulfoxide[37], the loss of a sulfonamide group[55] and the formation of an N-oxide[85], are examples of some new and unpredictable transformations. While work on the metabolic fate of new compounds may reveal some unique biochemically moderated reactions, it is less likely that any future study of drug metabolism will be of such fundamental importance to biochemistry as has been that of the acetylation of sulfonamides: a study which, by prompting the discovery of coenzyme A, has eventually led to the understanding of a substantial part of the intermediary lipid metabolism. However, the growing pool of basic knowledge of how drugs are metabolized will indoubtedly inspire more chemists to influence the metabolic fate of a drug by chemically or sterically modifying the anticipated sites of metabolic reactions.

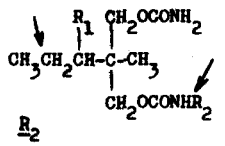
A major impetus to study the metabolic fate of a new drug comes from the growing number of drugs whose main pharmacological or biological activity is due to its metabolite(s). Some examples reported in 1964-65 include: hypotensive effect of N,N-diallymelamine due to its N-oxide[85]; hypoglycemic activity of 3,5-dimethylpyrazole due to the corresponding 3-carboxylate[82]; schistosomicidal property of "Miracil D" due to its 4-hydroxymethyl metabolite[101]; carcinogenicity of urethane[36], DAB[52] , and possibly of dimethylbenzanthracene[69] are due, in part, to metabolites.

Sufficient data are in the literature to foster the hope that eventually, for a given compound, it may be possible to predict the type as well as the rate of its metabolic transformation. However, variables imposed by nature such as species, sex, age, genetic background, etc., combined with those introduced by man, e.g. differences in experimental design, dosage schedules with the possibility of enzyme induction, etc., will continue to test both the ingenuity of the compiler and the capacity of his computer.

SOME METABOLIC TRANSFORMATIONS REPORTED IN 1964-65

COMPOUND	METABOLITES AND COMMENT
 Iodomethane	Metabolites are S-methylcysteine; methylmercapturic acid; methylthioacetic acid; N-(methylthioacetyl) glycine. Study showed metabolites of iodomethane and S-methyl-L-cysteine are the same, thus confirming S-alkyl-L-cysteine as intermediate metabolites in the metabolism of halogenoalkanes to alkylmercapturic acids (26).
Vasodilator  Nitroglycerine	Hydrolysis of a single nitroester (M) (A). Dinitro metabolites are resistant to further metabolism. Nitro-glycerin can react non-enzymatically with reduced gluthathione to liberate nitrite. However, a hepatic enzyme catalyzed reaction (27).
Vasodilator  Pentaerythritol tetranitrate*	Hydrolysis of all four nitro esters to pentaerythritol as only urinary metabolite (28). Follow-up study of same group who showed PETN is degraded stepwise in blood to the tri-, di- and mono-nitro ester and free pentaerythritol (29).
Anesthetic Hypnotic  Hydroxybutyric acid*	β-Oxidation (rat) (30). Findings indicate breakdown via β-oxidation rather than via Krebs cycle (terminal hydroxyl oxidation) (31); isolation of labelled hippuric acid after [4-^{14}C]- and not [1-^{14}C]hydroxybutyrate and Na benzoate; trapping of radioactivity with 3,4-dihydroxybutyrate, a suggested intermediate in β-oxidation. Y-Hydroxybutyrate reported as a normal constituent of brain tissue (31).
Sedative Mild hypnotic  Carbromal*	Only urea-C-atom containing metabolites were studied. 3-Hydroxylation, (M) mice; (m) rat, dog. Debromination. In vitro, with tissue slices, like in vivo, faster metabolism in mice than rats and dogs. Only debromination detected in rat and dog tissues. Median anesthetic dose (mg/kg) of carbromal, 3-hydroxycarbromal and 2-ethylbutyrylurea in mice (i.p.) were 256, 1000 and 506 respectively (32).

Tranquilizers

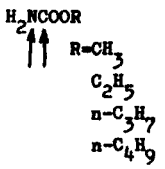


R_1	R_2	
CH_3	H	Mebutamate(33)
H	$CH(CH_3)_2$	Carisoprodol(34)
H	$(CH_2)_3CH_3$	Tybamate(35)

β-Hydroxylation(M); N-dealkylation(m); β-hydroxylation and N-dealkylation; conj.(gluc.)(tr).

Hydroxylation of β-carbon of propyl side chain is typical site of prevalent metabolic transformation of analogs of meprobamate. In vitro, β-hydroxylation not affected by α-methyl substitution (33). In spite of metabolic conversion to meprobamate, tybamate may act per se (35).

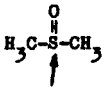
Carcinogen



Urethane and related carbamates

N-Hydroxylation, N- and O-acetylation(m), rat, rabbit, man. S-Carbethoxylation and S-ethylation followed by N-acetylation and S-oxidation reflected in isolation of S-ethylmercapturic acid, its sulfoxide and of N-acetyl-S-carbethoxycysteine. Same metabolites after N-hydroxyurethane.

Carcinogenicity of urethane may be due to its metabolism to hydroxyurethane which can cause biological carbethoxylation and ethylation (36).

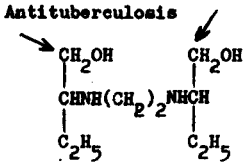


Dimethylsulfoxide

Reduction to dimethyl sulfide (cat) and dimethyl sulfone (rabbit) (37).

Reduction of an oxidized sulfur to a lower oxidation state in mammalian system is an unusual biotransformation.

Antituberculosis

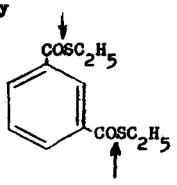


Ethambutol*

Oxidation of both alcohols to aldehyde (m) and subsequently to dicarboxylic acid metabolites (M); man, rat and dog metabolism similar (38)(39).

L and D isomers compared in dog and found L isomer quantitative conversion to metabolites greater than D isomer (40).

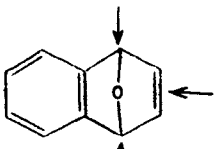
Antileprosy



Ditophal*

Ethylmercaptan (M), methyl ethyl sulfoxide (M) and methyl ethyl sulfone (m) formed.

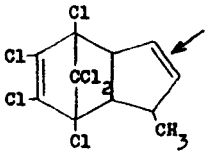
Used ^{35}S and only examined radioactive metabolites (41).



1,4-epoxy-1,4-dihydronaphthalene

Epoxide formed across double bond (M); epoxide at 1,4 transformed to a 1,4-$(OH)_2$ (m). Although related epoxy tetrahydronaphthalenes react with glutathione and are metabolized to mercapturic acid conjugation, this did not occur with this compound (42).

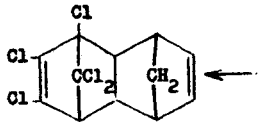
Insecticides



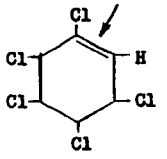
Heptachlor

Epoxide only metabolite formed in rabbit and rat microsomes.

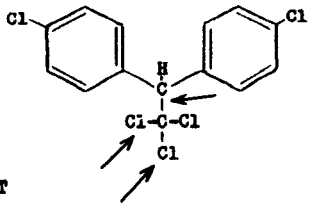
Cofactor requirements, O_2 and cellular localization of enzymes similar to other oxidative reactions on drugs. Lack of subsequent oxidation of epoxides to hydroxyl metabolites taken as evidence that epoxidation and hydroxylation are similar, but separate reactions dependent on the structure of the substrate (43).



Aldrin



Pentachlorocyclohexene

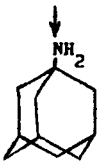


DDT

Dechlorination; dehydrochlorination (m); dechlorination and dehydrochlorination (tr); bis-dechlorination (tr); oxidative cleavage.

Established that, in rats, DDD, the dichloro analogue is, or can be, a metabolite of DDT. The suggested pattern of metabolic transformation of DDT is based on metabolic studies with metabolites of DDT (44).

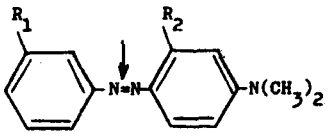
Antiviral



1-Adamantanamine

N-methylation only metabolite and occurs only in dog. Not metabolized in man, mouse or monkey (51).

Carcinogens*

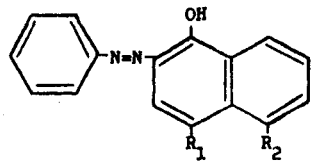


R_1	R_2
H	H
CH_3	H
H	CH_3

Reductive cleavage to amino- and diamino-metabolites; p-hydroxylation of the amino-metabolite; N-dealkylation.

In rats excr. of diamino metabolites may depend on their binding to tissue; this may vary with substitution. Carcinogenicity may be related to tissular binding of metabolite(s), e.g. DAB (R_1=R_2=H) and 3'-methyl-DAB (R_1=CH_3, R_2=H) are carcinogenic, 2-methyl-DAB (R_1=H, R_2=CH_3) is not (52).

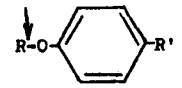
Dyes



R_1	R_2
H	SO_3Na
SO_3Na	H

2-Phenylazo-1-naphthol-4- and 5-sulfonic acids

Ring hydroxylation (M) and glucuronidation (M), no reduction of azo linkage (53).

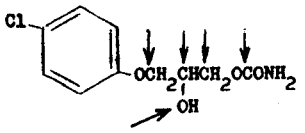


R
CH_3
C_2H_5
$CH(CH_3)_2$
$(CH_2)_2CH_3$
$(CH_2)_3CH_3$
$C(CH_3)_3$
CF_3

R' = NO_2 or $NHCOCH_3$

O-dealkylation primary metabolite, but does not occur if carbon atom adjacent to the oxygen is tertiary (54).

Muscle relaxant

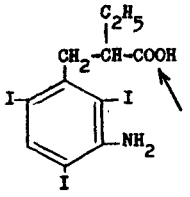


Chlorphenisin carbamate*

In rats (p.o.): UCC (6%); conj. (gluc. 49%; SO_4, 4%); hydrolysis and oxidation (17%); further oxidative cleavage (9%); O-dealkylation (5%). In man (p.o.): 85% excr. as gluc. with tr. of other metabolites.

Prolonged adm. or larger doses cause in rats metabolic tolerance. Carbamate group metabolically more stable in humans than rats (45).

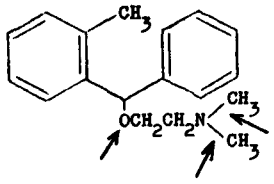
Radiopaque agents



Dopanoic acid

Glucuronidation in cats (only species studied). Found dopanoic acid and related compounds, tyropanic and bunamiodyl, form large amounts of glucuronides, with iodine atoms intact, and excrete in the bile (46).

Antiparkinson

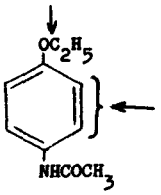


Orphenadrine

N-Desmethyl orphenadrine (M)

N-Bis-desmethyl orphenadrine (m), cleavage of the ether linkage of the amino alkyl chain (m) (47).

Analgesic

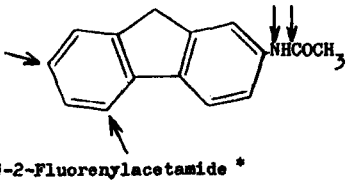


Acetophenetidine

In man (p.o.): isolation of new metabolites formed by O-dealkylation (M); conj. with cysteine (2%) and N-acetylation (tr). Also after p-acetamidophenol admin. Position of S-link not determined.

Low acetylating capacity of interest, since usually only observed in guinea pigs (48).

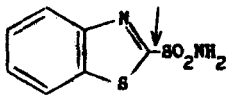
Carcinogen



N-2-Fluorenylacetamide *

Hydroxylation in 5,7 and on N; sulfate conjugation on OH at 7 (M); glucuronide conjugation on OH at 5,7 and on N (m); deacetylation. Extension of earlier work on metabolism of this carcinogen. Contrary to popular belief, the cat is capable of forming glucuronides as a minor metabolite(49). Hamster forms metabolites similar to other species (50).

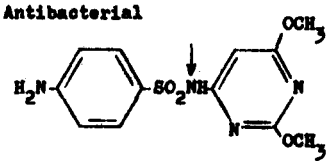
Carbonic anhydrase inhibitor



Benzothiazole-2-sulfonamide*

Complete cleavage of sulfonamide group and replacement with mercaptan (m), mercapturic acid (M) and mercaptoglucuronide (M). Reaction sequence initiated by the transitory metabolite glutathione conjugate (55).

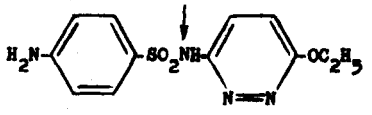
Antibacterial



Sulfadimethoxine

N'-glucuronide (M). Previous work showed formation of N-glucuronide, without determining on which nitrogen. This work established conclusively that conjugation occurs on sulfonamide nitrogen (56).

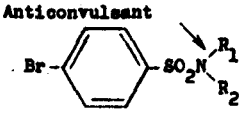
Antibacterial



Sulfaethoxypyridazine

N-4 Acetylated metabolite (M); small amount of glucuronide formed. Metabolism studied only in the heifer (57).

Anticonvulsant



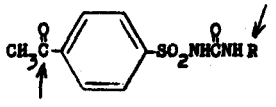
R_1= CH_3 to $(CH_2)_4CH_3$

R_2= H or CH_3

N-alkyl-4-bromobenzenesulfonamides

N-Dealkylation (M) (A); only the unsubstituted sulfonamide has activity. Dealkylation varies inversely with the bulk of alkyl substituent (58).

Hypoglycemic



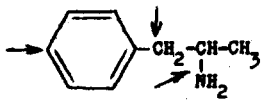
Acetohexamide (R= cyclohexyl)

U-18,536 (R= hexahydroazepinyl)

In acetohexamide: reduction of Ac to 1-CH_3CHOH (M)(A); hydroxylation of cyclohexane (m)(A)(59).

In man, both drugs are rapidly absorbed and rapidly transformed to p-α-hydroxyethyl metabolites, which remain hypoglycemic. Since the metabolites have a longer biological half-life than the parent drugs, their effect correlates well with the total serum sulfonylurea level (60).

Stimulant

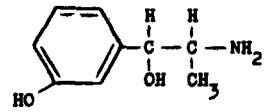


d-Amphetamine

Ring hydroxylation (m) rat, dog; gluc. conj. (M) rat, (m) dog; oxidative deamination; hippuric acid (M) monkey, dog;(m) rat.

This is a study comparing the metabolism in rat, dog and monkey (61).

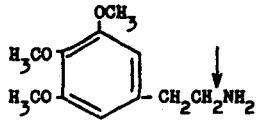
Sympathomimetic



Metaraminol

No metabolism occurs in rabbit liver homogenates capable of metabolizing tyramine, epinepherine, norepinepherine and amphetamine (62).

Hallucinogen

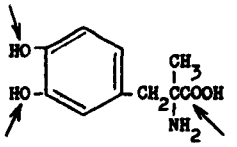


Mescalin *

In cats(63), and man(64): UCC (cat); oxidative deamination (M), cat, man.

Distrib. in cat brain(63) indicates binding to spec. intracell. components. Max. conc. of mescaline corresponded to approx. the estimated max. intox. Mescaline may be deaminated in brain. In man, trimethoxyphenylacetic acid (the major metab.) produced no definite physiol. or psychol. changes (64).

Antihypertensive

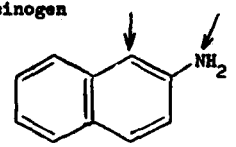


Methyldopa(l;d)

In rats (i.p.). l-Methyldopa and l-methyldopamine: UCC (M); O-methyl. (m); decarbox. (m); conj. (gluc.) (m). d-Methyldopa: only UCC and O-methyl. l-Methyldopamine: UCC, O-methyl. and conj. (gluc.).

l-Methyldopa better abs. from gut than d-; l-causes spec. aminoaciduria, possibly by competing for renal tub. reabs. of neutral amino acids; only l- is decarbox. These diff. may be responsible for therap. inact. of d- form (65).

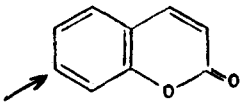
Carcinogen



2-Naphthylamine

Hydroxylation and sulfate conjugation at carbon 1 plus the unusual addition of a formyl group on the amino nitrogen (66).

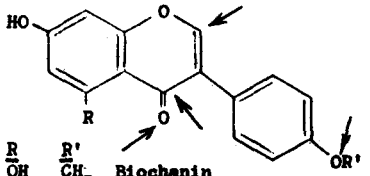
Anticoagulant



Coumarin

Detailed reexamination in several species of coumarin hydroxylation at C-7. Metabolite formed in rabbit and coypu in large amounts; cat, pigeon and guinea pig only 10 to 25% as much; no 7-hydroxylation in rat, mouse and locusts (67).

Natural estrogenic isoflavones

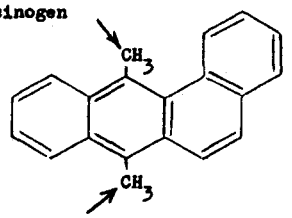


R	R'	
OH	CH_3	Biochanin
OH	H	Genistein
H	CH_3	Formononetin

In sheep, in this study, p-ethylphenol and p-cresol are major metabolites.

Only formononetin undergoes reduction of ring double bond and ketone (68).

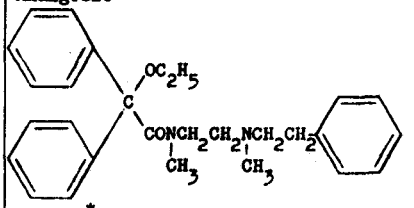
Carcinogen



7,12-Dimethylbenz[a]anthracene

Major metabolite is isomeric monohydroxymethyl compound. Minor metabolites include hydroxylation at 3 or 4 and dihydroxylation at 3,4 and 8,9. All metabolites appear to be carcinogenic (69).

Analgesic

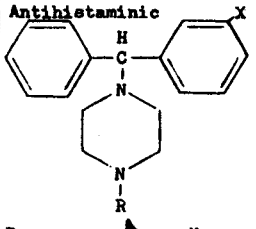


Bandol *

In rats, dogs: UCC (tr); metab. septd. by electrophor., not identified. Indicated: in vitro, primary and/or sec. amine formation; in vivo, ring hydroxylation and conj. (gluc.).

Radioact. mainly excr. in feces, notably in dogs. Metabolites similar in rats and dogs, differ in rel. amounts (70).

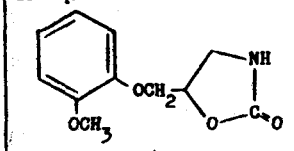
Antihistaminic



R	X	
CH_3	H	Cyclizine
CH_3	Cl	Chlorcyclizine
H	Cl	Nor-chlorcyclizine
3-methylbenzyl	Cl	Meclizine

N-Dealkylation (M). Norchlorocyclizine penetrates placenta (71) and may be cause of congen. malform. induced by meclizine and chlorocyclizine (72). Demethylation faster in male rats; slow in dogs, accel. by chron. admin. Chlorocyclizine is metab. slower than cyclizine, possibly due to plasma protein binding. N-demethylation causes loss of antihistaminic act. (73).

Tranquilizer

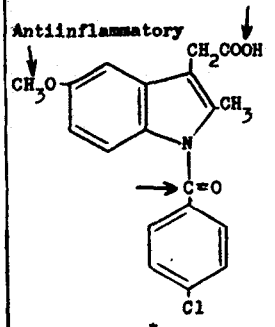


Mephenoxalone*

UCC (approx. 2%), dog; ring hydroxylation presumably at both p-positions, followed by conj.(gluc.)(M), dog, cat, rat, man.

In dogs, good intestinal abs. Unchanged drug is major plasma component up to 6 hr after dose. O-Demethylation not likely (74).

Antiinflammatory

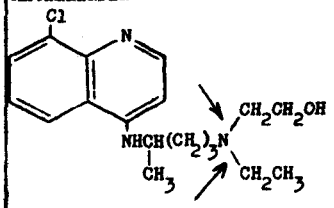


Indomethacin*

UCC (tr)(man); conj.(gluc.) of parent drug (M), man, monkey, rabbit, guinea pig, rabbit, rat, except dog; N-des-chlorobenzoylation(m); N-des-chlorobenzoylation and conj.(gluc.)(M); O-demethylation and conj.(gluc.).

Total gluc. excr. consistent and characteristic for species. Poor urinary gluc. excr. in rats and dogs. Renal excr. of indomethacin and metab. as gluc. is predominant clearance route (75).

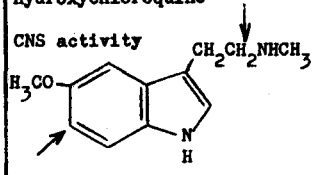
Antimalaria



Hydroxychloroquine

Studied only in rats; hydroxyethyl group removed (M) in preference to ethyl (m); trace amounts of free alkyl amino metabolite. No 4-amino quinoline detected (76).

CNS activity

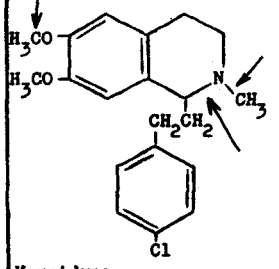


5-Methoxy-N-methyltryptamine

In rats; oxid. deamination (approx. 90%); postulated: 6-hydroxylation followed by conj.(gluc).

Isolated from the grass Phalaris tuberosa, causing "staggers" in sheep. In rats, the drug readily distrib.,rapidly and quant. metab. and excr. as 5-methoxyindoleacetic acid. N-Monomethyltryptamine remains substrate for MAO (78).

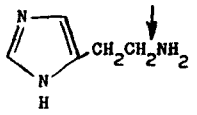
Analgesic



Versidyne

In rabbits, man: UCC (tr); 6-O-demethylation(M); N-demethylation(m); dehydrogenation(tr); conj. (gluc. 95%; sulf. 5%). Major metabolite, O,N-bis-desmethylversidyne (33-55% of dose).

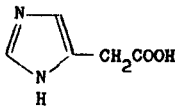
Fluorimetric det. of versidyne and metab. based on dehydrogenation with Hg-acetate (79).



Histamine

UCC (tr) mouse; oxidative deamination (m), followed by conj. (riboside)(M); rat, mouse. N-methylation (m), mouse.

In mice, circulating histamine is rapidly metabolized and its N-methyl metabolite detected. Both parent drug and its metabolite are stored in tissue depots. Imidazolylacetic acid and its riboside are major metabolites and also retained in tissues. The riboside predominated in the rat (80).

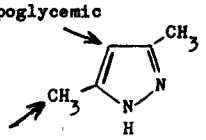


Imidazolylacetic acid*

No metabolites detected in rats in vivo (i.p.) or in vitro (liver slices and homogenates).

As reported (80), imidazolylacetic acid is metabolically inert, hence stable terminal metabolite of histidine and histamine (81).

Hypoglycemic

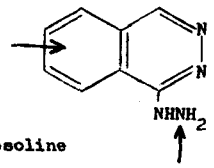


3,5-Dimethylpyrazole •

Oxidation of ring methyl to COOH (M)(A) with probable glycine conjugation.

Hydroxylation at C-4 and conjugation to produce major metabolite (70% of dose) (82).

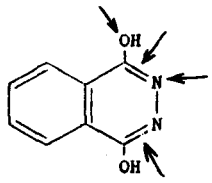
Hypotensive



Apresoline

In rats: UCC (15%); ring hydroxylation and conj. (gluc.) (40-50%); N-acetylation (25-30%); hydrazone of pyruvic acid (<5%).

LD_{50} (mg/kg) compared: apresoline, 35; hydrazone of pyruvic acid, 40; N-acetyl-apresoline, 100. No hydrazine liberated (83).

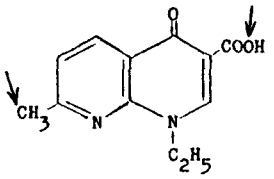


1,4-Dihydroxyphthalazine •

UCC, rat (30%), rabbit (45%); conj. (gluc.) (50%); N-methylation, rat (3.5%), rabbit (none detected); oxidative cleavage to phthalic acid, rat (7%).

O-Acetyl metabolites may have been formed but are readily hydrolyzed in the urine (84).

Antibacterial

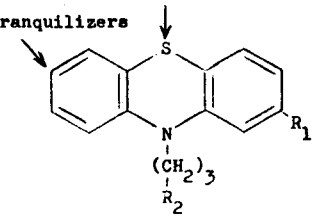


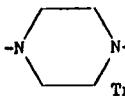
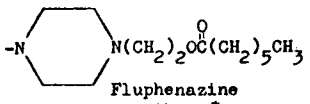
Nalidixic acid

In dog, monkey, man: UCC; conj. (gluc.) of parent drug (m); 7-methyl hydroxyl. (M)(A) with part. conj. (gluc.) and oxid. (tr.)

On chronic adm. of high doses (dog, monkey) no accum. of parent drug and/or metab(s). On bicarb. supplement., conj. of parent drug and its 7-CH_2OH metab.(I) is decreased. In man, I is excr. like parent drug, with less tend. to conj. In vitro, I is as antibact. as parent drug (94).

Tranquilizers



R_1	R_2	
H,	$-N(CH_3)_2$	Promazine
Cl,	$-N(CH_3)_2$	Chlorpromazine *
Cl,		Prochlorperazine*
CF_3,		Trifluperazine *
CF_3,		Fluphenazine enanthate *

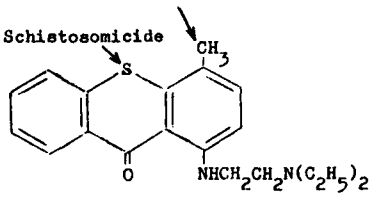
Chlorpromazine (I)(95): chron. adm. in man: UCC; 7-OH; S-oxid.; conj. (gluc.); N-demeth. (tr). Purple pigment. obs. in some psych. patients after high dose (96,97) may be light-induced alter. of 7-OH metab(s) enhancing tyrosinase act. and incr. melanin prodn.

In dogs, excr. is "side-chain" dependant. N-Methylpiperazinyl enhances biliary and reduces urin. excr. of parent cpds and/or their metabolites (98).

Fluphenazine (II)enanthate(99): hydrol. to II; S-oxid.; hydrol. and S-oxid.; CO_2 (tr). Excr. of radioact., urine 53%, feces 22%; bulk of tiss. radioact. not extracted. Prolonged act. due to slow release of ester from tiss. depots. Act. of ester due to II. Adm. as soln. in oil essent. for prolong. act., aqu. soln. short dur. of act.

Major metab. of promazine and I have N-(mono-, bis-) desmethyl. chain on hydroxylated ring and S-oxidn. (100).

Schistosomicide

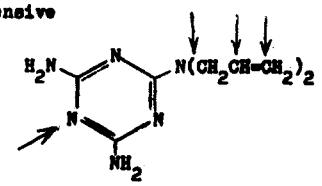


1-Diethylaminoethylamino-4-methylthiaxanthone hydrochloride

4-Methyl hydrox. (M)(A), in A.sclerotiorum; conj.(gluc.)(M), monkey; (m), mouse; S-oxid. (m), monkey; oxid. of 4-CH_2OH metab.(I) to aldehyde (m) in A.sclerotiorum.

In hamsters, I (hycanthone) has ED_{50} (p.o.), parent drug 8.0 mg/kg. Act. of parent drug apparently due to I, hence 4-methyl required for biol. act. In mice, the 6-chloro analogue also converted to 4-CH_2OH metabolite (101).

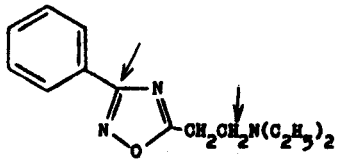
Hypotensive



N,N-Diallylmelamine *

Mono- and bis- N-dealkylation (M); dihydroxylation on chain double bond (M). N-Oxide in ring (unique biotransformation) is active hypotensive, formed in small quantity in rat and dog, but not in man (85,86).

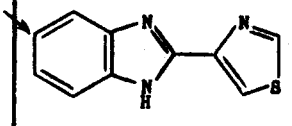
Antitussive



5-(2-Diethylaminoethyl)-3-phenyl-1,2,4-oxadiazole

UCC, rat (1-5%); deamination to diethylamine (rat, 10%; mice (tr); dogs, 5-12%) and to a neutral 3-phenyloxadiazole deriv. (rat, 1-5%; dog, tr). Oxidative ring cleavage to benzoic acid, conj. with glycine (rat, 5-15%; mice, dog, man) and gluc. (rat, mice, dog, man). Exploratory study (87).

Anthelmintic

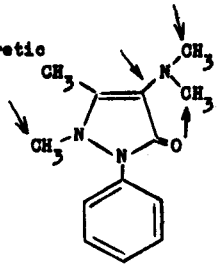


Thiabendazole*

In lambs(88): UCC (2%); 5-hydroxylation (M), only 10% unconj.; conj. (gluc. 70%, sulf. 14%). Similar findings in goats, calves and swine.

Rapidly abs., metab. and distrib. Only tr. detected after several days. Small amounts of metab. in milk of lact. animals disappear after several days (89).

Antipyretic

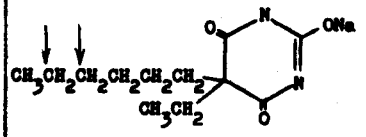


Aminopyrine

In dogs(90), man(90,91): UCC, man only; N-demeth.; N,N-bis-demeth.; N-acetyl. of N,N-bis-desmethyl metab.; deamin. of N,N-bis-desmethyl metab. to 4-OH deriv. Part. ring N-demeth. and intramol. cond. to rubazonic (I) and N-methylrubazonic (II) acid.

I and II inhib. ox. phosphor. more than indomethacin. But, in vivo less act. than parent drug (92).

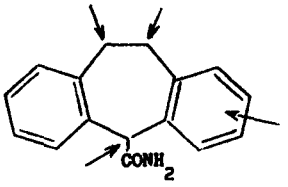
Sedative



5-ethyl-5-n-hexylbarbituric acid

Oxidation of C-5 of hexyl chain to OH,CO and COOH, as major metab.; also oxid. at carbons 4 (M), 3 and 2. Penultimate carbon predominant, but not exclusive site of oxidation (93).

Anticonvulsant

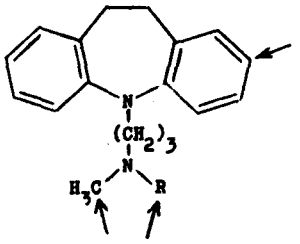


Dibenzo[a,d]cycloheptadiene 5-carboxamide

10-Hydroxylation; ring hydroxylation; 10,11-bis-hydroxylation; 5,10-bis-hydroxylation; conj. (mostly gluc.).

5,10-Bis-hydroxylation detected in rat and children; 10,11-hydroxylation predominant in rabbit and dog (102).

Antidepressants

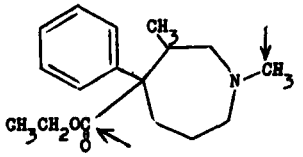


R	
CH_3	Imipramine
H	Desmethylimipramine

Rapid disappearance of both imipramine (I) and DMI (mice, rabbits). In rat and man I is metab. to DMI faster than DMI is metab. further. In vitro (liver microsomes): from rat, I demeth. mainly to DMI and 2-OH metab.; DMI metab. slowly. From rabbit, DMI metab. faster than I; N-demeth. minor pathway. From human, slow metab. of I and still slower of DMI.

Species variation as obs. in the pharmacol. response of I and DMI may reflect metab. fate which differs from species to species (103).

Analgesic

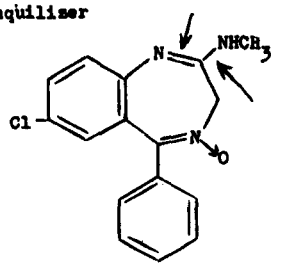


Wy-535 (5-methyl-ethoheptazine)*

In dogs: UCC (10%); ester hydrolysis (60%); N-demethylation (30%). N-Demethylation greatly accelerates ester hydrolysis.

Radioact. excr. mainly in feces in rats (50%), but urine in dogs (73%). Unlike with ethoheptazine (104), no hydroxylated metab. detected: implied interference by 3-methyl suggestive of 2- or 3-hydroxylation of ethoheptazine (105).

Tranquilizer

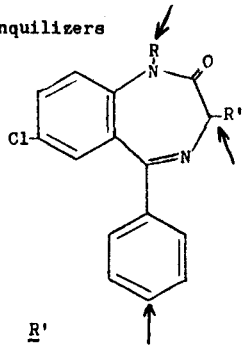


Librium

Hydrol. cleav. to 2-oxo metab. ("lactam") (M) (A), dog, man; further cleav. to "opened lactam" (m), dog, man; metab. of "opened lactam" to alkali-sens. undef. "conj." (m), man. Rat: unident. basic metabolite (M) (106).

Librium is extens. metab. in rat, dog, man. Major metab. pathways similar in dog, man, diff. in rat. Major site of metab. transform. is N_1-C_2 link. N-oxide or benzophenone moiety not changed. "Lactam" centr. as act. as librium in mice (anti-fight. test, anticonvuls. and muscle relax. effects), but inact. in cond. avoid. behaviour (rat) (107).

Tranquilizers



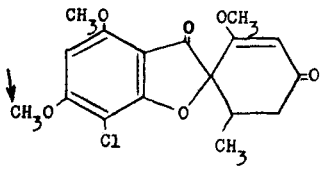
R	R'	
CH_3	H	Diazepam
H	OH	Oxazepam *
H	$OCCH_2CH_2COH$ (O, O)	Oxazepam succinate *

Diazepam(I): N-demeth.(tr)(A); N-demeth. and 3-hydrox.(tr)(A); N-demeth., 3-OH and conj.(gluc.)(M). Blood metab. (dog, man) show rapid form. of N-desmethyl-I (half-life longer than I). In dogs, oxazepam (II) gluc. is major metab. of I and II. In man, gluc. of 3-OH-I and II det. in urine(108).

In man, stored I and metab. are released slowly (109). N-Demeth., p-OH and conj. (gluc.) (rabbit) (110). N-Demeth. increases antiagress. eff. (monkey); 3-OH enhances anticonvuls. and muscle relax. eff. (mice). Pharmacol. act. of parent cpd not due to metabs. (107). Dur. labour I was in maternal and fetal circul., tr. in amniotic fl. (111).

Oxazepam and succinate metab. similar in dog, pig, man: mainly conj. (gluc. 95%). Complex in rat (112).

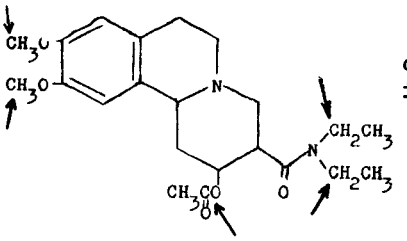
Antifungal



6-Demethylation followed by conjugation to produce major metabolite. A second metabolite in some conjugated form not identified (113, 114).

Griseofulvin *

Psychotherapeutic agent



N-Desethylation (M), O-demethylation (m), deacetylation (m), a total of 11 metabolites identified in urine of dog and man (115,116).

Benzquinamide *

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Chapter 24. Regulation of Cell Metabolism

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This review will consider recent highlights in two areas of metabolic regulation: 1) Inhibitors of nucleic acid replication, transcription and translation processes, thought to act directly at the enzyme level, and 2) hormonal regulation of cellular metabolism at nucleic acid and protein synthesis sites from the point of view of the medicinal chemist who is seeking to apply this knowledge in the development of new and useful drugs.

During the past two years, many publications on the mechanism of replication of the genetic material deoxyribonucleic acid (DNA), its transcription to yield specific ribonucleic acids (RNA) and the translation of RNA to give specific proteins have appeared. A wide variety of agents have been reported to affect various steps in these sequences. Thus, ultraviolet irradiation[1, 2], acridine dyes[3, 4], the antibiotics phleomycin[4, 5], novobiocin[6], actinomycin D[3, 4, 89, 108], nogalamycin[4, 7], chloramphenicol[8-13, 107], streptomycin[14-17], erythromycin[18, 19], puromycin[4, 20-22], gougerotin[23, 24], pluramycin[25], daunomycin[4, 26], miracil D[27, 28], chromomycin[4, 29], tetracyclines[30-33], cycloheximide[4, 34-37], mikamycins[35], actinospectacin[36], cycloheximide analogues[38], rifamycin[39], mithramycin[40], mitomycin and porfiromycin[41-43], streptomycin-like antibiotics[17], edeine[51], peptide antibiotics[52], cinerubin[4], oliomycin[4], naladixic acid[106], nucleosidin[112], estrogens[44-47, 104], corticosteroids[47-50, 55, 56, 104], ecdysone[47-p.142], androgens[46, 47, 53, 54, 104], thyrotropic hormone[57, 58], histones[61-69], RNA[70, 71], ACTH[59, 60], thyroxine[47, 72, 104], vitamin D[73, 74], para-thyroid hormone[75], growth hormone[76, 77], insulin[47, 104], Vinca alkaloids[78], aldosterone[79], vitamin K[80], aflatoxin[36a, 81], and alkylating agents[82, 83] have all been reported to act either directly or indirectly at some site in the control of DNA, RNA or protein metabolism. Since DNA is the genetic material which determines the nature of the cell (genotype) and RNA may be looked at as the photocopy thereof which determines phenotypic expression, we are indeed dealing in these processes with the most basic controls of cellular function. Several interesting review articles or symposia have been published in the recent past which summarize basic concepts and developments in this exciting field[4, 47, 84-88, 90].

Direct Inhibitors of Nucleoprotein Metabolism at the Macromolecular Level

A simplified schematic diagram of the replication of DNA and the transcription thereof to RNA which in turn dictates cellular protein is shown in Fig. 1. The proposed sites of inhibition by selected agents are also shown in this figure and will be described in some detail below.

Inhibition of DNA replication. - Inhibition of DNA replication can be effected by agents which inhibit the DNA polymerase enzyme *per se* or agents which affect the primary or secondary structure of DNA such that it can no longer interact properly with the enzyme. One example of the former type of inhibitor may be the antibiotic novobiocin[6], while inhibitors of the latter type include ultraviolet and x-irradiation[1, 2] and the antibiotics actinomycin D[3, 4], nogalamycin[7], phleomycin[4, 5], mitomycin C and porfiromycin[41, 43].

One common feature of the latter inhibitors involves reaction with DNA such that the separation of the strands of this double helical molecule, which is

required for its replication by DNA polymerase[91] is inhibited. Thus, ultraviolet light causes dimerization of thymine residues which may crosslink the molecule and prevent strand separation[1]. The sites of action of actinomycin D and nogalamycin are discussed below. Phleomycin binds to the DNA primer at the A-T moieties and prevents its interaction with DNA polymerase[4, 5]. The structures of these antibiotics are not presented here but can be seen in the reviews by Goldberg[4] and Umezawa[92]. Clearly these agents are not related chemically to one another and in fact insufficient chemical similarity exists to draw any structure-activity relationships. Other antibiotics which combine with DNA or inhibit DNA polymerase include chromomycin[29], streptonigrin[4], and edeine[4], which are discussed in more detail in the review by Goldberg[4].

Inhibitors of DNA transcription. - Three noteworthy inhibitors of this site of metabolic activity are actinomycin D[3, 4], nogalamycin[7] and daunomycin[28]. The inhibition of DNA replication by actinomycin and nogalamycin is a secondary site of action observed at high concentration. Time-course inhibition and dose-response studies have shown that the primary site of action for both of these agents is the inhibition of RNA synthesis, which is followed by DNA and protein inhibition in mammalian cells[4, 7].

Actinomycin D is known to inhibit RNA synthesis by binding to the guanine residues of DNA where it fits into the minor groove, thus inhibiting the function of RNA polymerase which apparently also occupies this position[4, 93, 94]. Nogalamycin also shows base specificity but appears to bind preferentially to the adenine or thymine portions of DNA, or possibly both, thereby inhibiting the RNA polymerase reaction[7]. It is interesting to note that actinomycin binds specifically to a base in DNA but does not intercalate, whereas nogalamycin shows evidence of intercalation in addition to base specificity. Thus, the latter antibiotic, although it contains only one nitrogen atom*, increases the viscosity of DNA markedly and its binding to DNA is reversed by salts[7], properties common to the acridines[4, 95]. On the other hand, it shows strict base specificity under the experimental conditions used, both with respect to physical chemical changes of the DNA and its reaction in the RNA polymerase system[7].

Although direct structure activity correlates cannot be made at this time with respect to predicting base specificity or intercalating ability of these molecules, certainly considerable rational effort can now be directed at the question of modifying the interaction of such agents with the DNA primer in order to increase or alter base specificity, salt displacement reactions, and so forth. Likewise, altering organ specific absorption of these agents may indeed also lead to unique chemotherapeutic effects, impossible to obtain with the present compounds.

Inhibitors of RNA translation. - Several antibiotics have been shown to inhibit protein synthesis by interfering with some step in the DNA-RNA-protein sequence subsequent to RNA synthesis. Chloramphenicol is reported[8-13] to inhibit the attachment of messenger-RNA to ribosomes. This is a fascinating concept since it can also explain the specificity of chloramphenicol in inhibiting bacteria. The life span of messenger-RNA in bacteria is measured in minutes[96], while that in mammalian cells is measured in hours[97]. Thus, in the relatively short period in which significant concentrations of chloramphenicol are present in

*R. B. Kelly and P. F. Wiley, private communication

an animal after discreet dosing, sufficient antibiotic may be expected to be absorbed by the bacteria to seriously impair their ability to synthesize protein because of the rapid turnover of the messenger-RNA and its required regeneration. A mammalian cell on the other hand with a much slower turnover would be expected to be less inhibited. When sufficient antibiotic is given over an adequate period of time, toxicity and cytotoxicity are indeed observed with chloramphenicol even in animal cells[98].

Streptomycin causes misreading of the m-RNA after the latter is bound to ribosomes to form a polysome, and other antibiotics of this chemical type appear to act in the same way in vitro[14-17]. Cycloheximide inhibits protein synthetase, which catalyzes the formation of the peptide bond, while puromycin substitutes for a molecule of transfer-RNA and accepts the growing peptide following which it uncouples the system and releases nascent peptide into solution[4, 34-37].

Again, no common chemical characteristics are evident which would suggest a priori to a knowledgeable investigator that a particular activity of the type observed should be possessed by that molecule. Knowing the structures of these substances[4] and having available enzymatic systems which are thought to represent their primary sites of action, however, gives the medicinal chemist a very powerful tool with which to investigate analogues and derivatives for increased specificity or selectivity of action, increased or decreased binding capacity, and the like. Comparative studies of compounds which prevent the attachment of m-RNA to ribosomes in systems with short-lived vs. those with long-lived messengers hopefully will lead to compounds with the potential of chloramphenicol for antibacterial activity. A common feature of all of the inhibitors discussed thus far is the direct action which they can be shown to possess either on the specific primer or enzyme involved in the reaction. This represents a boon to the medicinal chemist who is interested in structure-activity relationships, since he can test directly for the effects of analogues and derivatives of given compounds on such parameters as DNA melting, intercalation, effects on RNA and DNA polymerases, peptide synthetase, combining of messenger to ribosomes, etc. Thus far, the new molecular species showing the selective inhibitions reviewed above have originated from the empirical approach of antibiotic screening, which needs no justification for continued exploitation. It is stimulating to speculate on the ability to design even more potent or more selective agents in the future, making use of the structure-activity relationships already known. It is interesting that certain of these inhibitors, particularly those binding to DNA (some of which cause damage which requires a certain degree of repair by the organism), are highly toxic to animals and other forms of life as well (irradiation, actinomycin D, nogalamycin and mitomycin). Certain of the other agents, however, are capable of exerting profound effects against microorganisms with low orders of toxicity to animal cell (chloramphenicol, streptomycin, erythromycin and novobiocin). Continued studies on the mode of action of various agents inhibiting different sites in the pathway from DNA to protein may allow the medicinal chemist in the not-too-distant future to determine whether the inhibition of a certain step in this process is likely to lead to selective toxicity to one form of organism rather than others. Such conclusions, however, must await the discovery or synthesis of additional inhibitors known to act at specific sites in the above pathways.

Hormonal Controls

Steroids. - A series of papers appeared in 1965 on the mechanism of action of corticosteroids, androgens and estrogens[44-50, 53-56, 99]. Various studies published in recent years have demonstrated the induction of a variety of enzymes in liver (glucose-6-phosphatase, fructose-1,6-diphosphatase, phosphohexose isomerase, PEP-carboxykinase, tryptophane pyrrolase, transaminases, arginase, glutamic dehydrogenase, arginino-succinase and arginine synthetase) after administration of corticosteroids to animals[100, 99-p. 1, 101]. Synthesis of these enzymes is preceded by synthesis of RNA, also elicited by corticoid administration[84-p. 125]. Early studies[100] demonstrated the induction of tryptophane pyrrolase and tyrosine-α-ketoglutarate transaminase by cortisone or substrates, administered independently to adrenalectomized animals. Since puromycin inhibited the induction of these enzymes by steroids or their substrates, protein synthesis was presumed necessary. Actinomycin on the other hand was shown to abolish the cortisone mediated induction, but not the tryptophane induced enzyme activity, thus differentiating between these processes and suggesting that the steroid induction of enzyme, but not that of substrate, was mediated by the synthesis of m-RNA.

Detailed studies by Weber and co-workers[99-p. 1] on the induction of hepatic gluconeogenic enzymes by corticosteroids led these authors also to conclude that the process involves de novo protein synthesis and that it is sensitive to puromycin, actinomycin and ethionine. In view of the fact that the gluconeogenic enzyme level is also elevated in the liver of chronic alloxan diabetic rats, and the observation that actinomycin D and puromycin did not inhibit this increase in enzyme, these investigators postulated that the insulin molecule may act as a physiological repressor of the synthesis of gluconeogenic enzymes and that the corticoids act by reversing this repression. Investigations by Kenney and collaborators have demonstrated an increased synthesis of nuclear RNA under stimulation in vivo by hydrocortisone[99-p. 125]. The stimulation of RNA synthesis by hydrocortisone was also inhibited by actinomycin D. The conclusion from these investigations is that the steroid hormone induces the synthesis of RNA which in turn results in an increase in the levels of specific enzymes. Whether this is indeed the primary, rather than a secondary, effect of the steroids cannot be answered at the present time, although some investigations suggest that the latter may be the case[101, 99-p. 137]. Regardless of whether the effect is primary or secondary, however, studies on the structure-activity relationships of the various hormones with respect to nucleic acid and enzyme induction in specific tissues and the prevention thereof by inhibitors with known sites of action might provide a new basis for the synthesis of analogues or the separation of biological activities that could result in more favorable therapeutic applications of the steroid molecule.

Similar types of investigations with androgenic hormones demonstrate an increased level of RNA polymerase in prostate following administration of testosterone to castrated animals as well as an increased capacity for amino-acyl-t-RNA synthesis and amino acid incorporating capacity of ribosomes in vitro[84-p. 11]. These early changes in nucleic acid polymerase activity are accompanied in vivo[46-p. 22] by increases in soluble proteins, activities of proteases, and α-amylases and actual weight of prostatic and seminal vesicle tissue. All of these changes are blocked by the administration of actinomycin D and can only be observed after administration of the steroid to the animal. No effect on RNA polymerase activity has been observed upon addition of

testosterone or other sex hormones to the enzyme system in vitro.

In the case of estrogen action on the uterus of castrated animals, the data are among the most convincing for a direct and probably primary effect of the hormone on the induction of protein and RNA synthesis. Detailed studies by Jensen[86-p. 317], Gorski[84-p. 91] and their co-workers have demonstrated the binding of estrogen to the uterus of the castrated rat with little or no detectable metabolism following this event. Mueller and co-workers[47-p. 228] demonstrated increased rates of protein and RNA synthesis as early as six hours after estrogen injection with RNA accumulation beginning at approximately 12 hours and protein accumulation at 24 hours. No increase in DNA synthesis was seen even at 24 hours after administration of the hormone. The early changes in RNA and protein synthesis were shown to be blocked by puromycin, actinomycin, and cycloheximide[47-p. 228, 84-p. 91], indicating that the initial effects of estrogen are dependent upon RNA and protein synthesis. Gorski[84-p. 91] also demonstrated an increase in uterine RNA polymerase after treatment of the animal with 17-β-estradiol. Based on investigations of early effects of inhibitors, Mueller and co-workers[47-p. 228] conclude that the initial step in the action of estrogen is the activation of a protein which in turn stimulates synthesis of RNA polymerase, RNA, and subsequently the various proteins which comprise total uterine proliferation.

As noted by Gorski et al.[84-p. 91], these facts are consonant with the molecular requirements for action of any hormone: 1) An interaction of the hormone with a receptor molecule of the target cell, 2) initiation of the primary response of the tissue to the hormone, and 3) amplification of this response by secondary effects to give rise to the ultimate expression of hormone action. As was emphasized by Gorski[84-p. 91], it must be remembered that most of the evidence to date, bearing on the question of primary or secondary response of mammalian cells to steroid hormones at the molecular level, does indeed rest on studies with inhibitors rather than direct measurements. However, this does not detract from the potential significance of the effects observed on the enzymes of nucleic acid metabolism, e.g., the increased synthesis of nucleic acid per se and ultimately of organ specific proteins, or the potential application thereof in testing for new drug effects in simplified in vitro systems. Such screens might indeed result in the discovery of agents with unique biological properties which would go undetected in the standard whole animal model systems.

Protein hormones. - Studies of the type mentioned above performed by Wool and collaborators[47-p. 98] have demonstrated the ability of insulin to induce early RNA and protein synthesis in muscle. In a careful series of experiments, these investigators show that the induction of nucleic acid and protein synthesis could not be reproduced by simple increased penetration of glucose or amino acids into the cells under the influence of this hormone. They further showed that the inductions by insulin were quantitatively markedly reduced when actinomycin D was administered in vivo although the percentage stimulation over the control by insulin was still noted in the presence of the inhibitor.

Several recent studies[99-pp. 151, 177, 189] clearly demonstrate the induction of glucokinase in rat liver by insulin. Although other factors such as glucose, thyroid and pituitary hormones may also play a role, insulin is indeed

a major factor in this induction. Weber and co-workers showed that insulin also functions as an inducer of the other two key hepatic glycolytic enzymes: phosphofructokinase and pyruvate kinase[113, 114]. The insulin dose-dependent induction of the three hepatic key glycolytic enzymes[115] and recently also that of glucose 6-phosphate dehydrogenase was described[116]. Inhibitor studies again demonstrate that de novo protein synthesis is involved as well as RNA synthesis in the induction of these enzymes. Similar studies by Benjamin et al.[102] on the induction by insulin of enzymes required for fatty acid synthesis in liver again suggest that de novo synthesis of protein is involved in this process, preceded by RNA induction. Ilyin[99-p. 151] has further demonstrated a direct antagonistic effect by certain hormones on specific enzymes (e.g., hexokinase antagonism by corticosteroids and reversal by insulin). Clearly the effect of insulin on glucose penetration in muscle is not mediated by de novo protein synthesis[103]. Weber and co-workers[99-p. 1] suggested that insulin may act as a physiological suppressor of hepatic gluconeogenic enzymes. Thus, the above studies on insulin strongly suggest a multiplicity of effects, some of which are instantaneous and do not involve de novo protein or RNA synthesis (e.g., glucose penetration, activation of hexokinase in the presence of steroid), while others occur more slowly and appear to be mediated by de novo synthesis of RNA and protein (e.g., induction of enzymes for glucose utilization and fatty acids synthesis). As in the case of the steroids, it is impossible at this point to state with certainty whether these represent primary or secondary results of hormone treatment, but it is clear that increased penetration of amino acids or glucose cannot account for the results observed. Wool[47-p. 98] raises the possibility that insulin increases the rate of transport of m-RNA from the nucleus to the cytoplasm as a primary event, followed by a secondary stimulation of nuclear RNA synthesis. Again, whether primary or secondary, the increased synthesis of m-RNA appears to be necessary to maintain a sustained effect of insulin on the induction of enzymes in the glucose and fatty acid pathways.

In addition to studies with insulin, similar investigations, again relying heavily on the use of inhibitors, have suggested that a critical site of action of growth hormone[84-p. 153] and certain other peptide hormones[57-60] is the induction of de novo RNA and protein synthesis which ultimately results in the expression of their hormonal activity at the organ level. Studies by Tata and co-workers[47-p. 173, 109] on the mode of action of thyroid hormone demonstrate an interesting sequence in which the induction of RNA polymerase precedes the production of RNA per se, which in turn precedes protein synthesis and tissue growth. Such data are impressive from the standpoint of the required sequence for ultimate expression of hormone effect.

General Summary

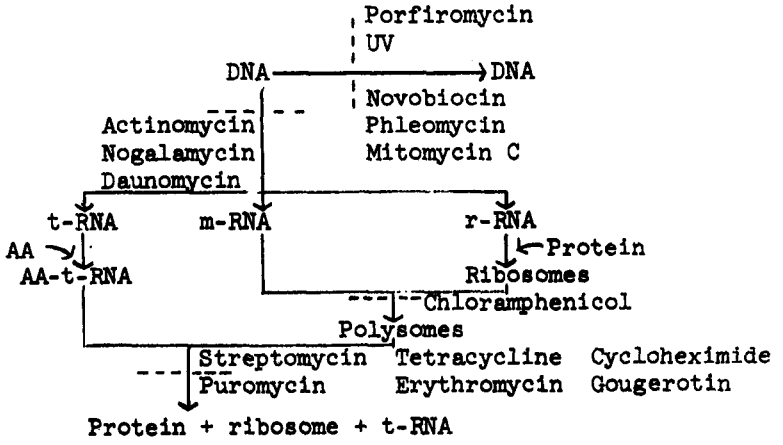
Incontrovertible evidence is now available in the biochemical literature demonstrating the significance of 1) DNA polymerase and double-stranded DNA in the replication of the genetic material and 2) RNA polymerase in the transcription of DNA to various classes of RNA which in turn are translated via an elaborate mechanism to synthesize specific proteins. A variety of small molecules, notably antibiotic substances, have been shown to influence specific sites in these sequences, both in vitro and in whole organisms. The concentrations which are effective in vitro can be expected in many cases to be realized in vivo and likely do account for the primary biological effect observed with these agents. Studies with the corticosteroids, androgens and estrogens, as well as histones, protein hormones, thyroxin and vitamin D have implicated these agents as inducers of de novo protein synthesis in a variety of test situations either by direct stimulation of DNA transcription or RNA translation. Obviously, all

of the data collected are not completely consonant but a general summary of these investigations does favor the concept that some of these hormones induce the synthesis of RNA polymerase which in turn results in an increased rate of synthesis, and in some cases accumulation of, the various classes of RNA and ultimately of tissue-specific protein.

Whether the effects on RNA synthesis are the primary ones of the hormones or secondary to some other more basic molecular action such as effect on permeability or translocation of RNA cannot be concluded from the data at hand. However, it is evident that 1) the effects are real, 2) they can be observed *in vitro* and *in vivo* and 3) the expression of the hormone-specific action in the target tissue is blocked by specific inhibitors of RNA and protein synthesis. It is of further interest that, in most cases, the hormonal effects on nucleoprotein metabolism cannot be demonstrated directly at the enzymatic level *in vitro* as can the inhibitory effect of the antibiotics. It is also evident from the available studies that certain of the hormone effects which appear to be instantaneous are not mediated by RNA and *de novo* protein synthesis (e.g., glucose penetration and insulin, fat mobilization and protein hormones, etc.) whereas the induction of enzymes and certainly the growth of tissue as the result of hormone administration are mediated by *de novo* macromolecule synthesis.

Whether in the last analysis the effects observed to date are found to be primary, secondary or even further removed from the initial molecular effect of the hormone, the data in hand clearly point to the DNA transcription and RNA translation series of reactions as having profound importance in metabolic regulation mediated through hormones and antibiotics. In this regard, with the availability of purified enzymes, nucleic acid primers and means to measure products of these reactions, the medicinal chemist should look with renewed enthusiasm at the structure-activity studies in the hope of 1) divorcing multiple effects of hormones, 2) discovering new activities in previously unsuspected molecules, or 3) splitting activities in known types of chemical agents. The possible use of selective inhibitors to separate overall metabolic expression of drug or hormone effects *in vivo* should not be excluded. Likewise, concentration of agents in specific organs or selective binding at various sites within the cell, and competition for binding by related or unrelated molecules must be considered in the overall picture of selective drug effects in the intact animal and approaches open to the medicinal chemist seeking new chemotherapeutic agents.

Figure 1.



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Chapter 25. Agents Which Affect Enzyme Activity

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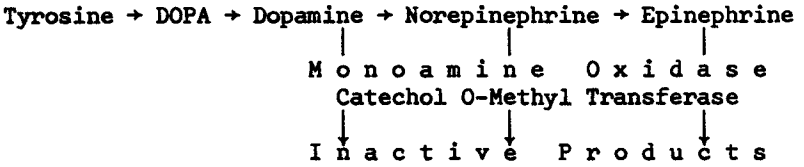
The discovery of numerous drugs acting on specific enzyme systems has resulted in the rapid development of biochemical pharmacology. This is evident from the newer classifications of drugs such as the inhibitors of carbonic anhydrase, choline esterase, and monoamine oxidase. Probably an even greater number of agents act on enzyme systems, or at least at the biochemical level. However, our lack of knowledge of detailed biochemical mechanisms prevents us from describing their actions on specific enzyme systems. Such phenomena as drug effects on transport mechanisms, on uptake and release of transmitters, and on contractile mechanisms most likely occur by drugs acting on enzymes. The biochemical mechanisms involved in these events are not well understood, but newly acquired evidence points to the involvement of enzymatic processes. Eventually it may be possible to explain many, although by no means all, heretofore unknown mechanisms at the biochemical level. Several excellent reviews on the effect of drugs on enzyme or biochemical systems have been written earlier.[1,2]

This report also discusses drug effects on enzyme systems, but a comprehensive coverage of the known enzymes is not feasible. Rather, the pertinent literature from late 1964 through 1965 will be reviewed in the selected area of drug effects on enzyme systems which are concerned with the metabolism of several of the biogenic amines. This area was selected because it represents some of the best examples of current thought on the biochemical approach to pharmacology.

INHIBITORS OF CATECHOLAMINE METABOLISM

The pathway of epinephrine and norepinephrine metabolism is now well established. Both amines are derived from the amino acid tyrosine in the following sequence:



Tyrosine Hydroxylase Inhibition

In the above metabolic scheme of catecholamine formation the conversion of tyrosine to DOPA by tyrosine hydroxylase represents the rate limiting step.[3] The properties of this enzyme were described in detail by Nagatsu, Levitt and Udenfriend[4,5] who found it distributed in various organs and associated with particles sedimenting at 15,000 x g. In beef brain tyrosine hydroxylase is concentrated in the caudate nucleus; this is of interest since the caudate is thought to contain the adrenergic synaptosomes.[6] Because of its relatively low activity *in vivo*, tyrosine hydroxylase appears to be an ideal site of action

for drugs to inhibit synthesis of norepinephrine. Already several agents have been discovered that act as inhibitors of tyrosine hydroxylase. Compounds described as inhibitors of purified beef adrenal tyrosine hydroxylase include α-methyl tyrosine, 3-halogenated tyrosine derivatives, mono- and di-iodotyrosines, and several catechol compounds, especially 3,4-dihydroxyphenylpropylacetamide (H 22/54). The mechanism of inhibition of H 22/54 appears to be a competitive action with the cofactors of tyrosine hydroxylase, since it was reversed by dimethyltetrahydropteridine. The anti-enzyme effect of 3-iodo-L-tyrosine *in vivo* was also investigated,[8,9] and a decrease in norepinephrine of certain tissues as a result of tyrosine hydroxylase inhibition in the intact animal has been demonstrated. The latter investigators also found that in rats, the pressor response to the indirect acting sympathomimetic agent, tyramine, was markedly attenuated after the administration of 3-iodotyrosine. This tyramine blockade may be presumed to be the result of the diminished stores of tissue norepinephrine.

Equally interesting as a tyrosine hydroxylase inhibitor is the compound α-methyl-p-tyrosine (MPT). Unlike the α-methyl-m-tyrosine (MMT), MPT does not cause a release of endogenous catecholamines, nor is it decarboxylated to form α-methyl-p-tyramine.[10] The compound causes a lowering of tissue catecholamines by inhibiting tyrosine hydroxylase and not by displacement of endogenous amines as in the case of the meta-hydroxylated congener. As expected, MPT also prevented the rise in tissue catecholamines after administration of the MAO inhibitors.

The preliminary pharmacological properties of MPT are also of interest in that certain reserpine-like effects were demonstrated. This agent may be a valuable aid in clarifying the much debated mechanism of reserpine. Weisman and Koe[11] found that MPT was also able to block the stimulant properties of amphetamine, but potentiated the avoidance-disrupting properties of chlorpromazine. They interpreted these results to mean that, at least in part, amphetamine exerted its central effect indirectly via levels of free norepinephrine.

In man MPT decreased catecholamine synthesis in subjects with pheochromocytoma or essential hypertension as measured by changes in urinary metabolites.[12] A fall in blood pressure was noted in the pheochromocytoma patients, but not in those with essential hypertension. However, in all cases sedation was present.

Drugs such as MPT have potential not only as powerful tools for investigating the biochemical steps in catecholamine synthesis and regulation, but also as possible therapeutic agents in disorders of catecholamine biochemistry. However, one needs to temper this enthusiasm until further investigations confirm the specificity of this inhibition. It is possible that other metabolic pathways of tyrosine may also be affected and could lead to other complications.

DOPA Decarboxylase Inhibition

The conversion of DOPA to dopamine is catalyzed by DOPA decarboxylase, a pyridoxal dependent enzyme possessing a rather broad substrate specificity. This last property has resulted in the proposal of the alternate nomenclature of "aromatic amino acid decarboxylase," a term which is appropriate in view of its ability to decarboxylate o- and m-tyrosine, DOPA, and 5-hydroxytryptophan,[13] and possibly several other aromatic amino acids.

Over the past decade a considerable number of DOPA decarboxylase inhibitors have been described, but in no instance has it been possible to produce adequate inhibition *in vivo* to cause depletion of tissue catecholamines. The earlier view of the mechanism of action of α-methyl DOPA and α-MMT acting via decarboxylase inhibition is no longer tenable. These α-methylated amino acids are inhibitors of amino acid decarboxylase, but they themselves are also decarboxylated to varying degrees, resulting in the formation of α-methyl dopamine and α-methyl-m-tyramine, respectively. These amines then become hydroxylated at the β-carbon, and because of their chemical similarity to norepinephrine, displace the sympathetic mediator in the adrenergic nerve granules. In this location they act as norepinephrine substitutes and may be released by nerve stimulation and may or may not be released by certain pharmacological agents.[14-18] Thus, the anti-decarboxylase activity of these α-methylated amino acids plays an insignificant role in the pharmacological and biochemical actions that they produce. Another difficulty in evaluating the decarboxylase inhibitor activity of these agents is stressed by Moran and Sourkes[19] who found that they inhibited several enzymes involved in amino acid metabolism.

Since the finding of the anti-decarboxylase activity of the α-methyl amino acids, more potent and specific agents have been described. Notable among these are N-(3-hydroxybenzyl)-N-methylhydrazine (NSD 1034) and N-(DL-seryl)-N'-(2, 3,4-trihydroxybenzyl) hydrazine (RO 4-4602). Both of these compounds are able to prevent the conversion of administered DOPA to dopamine, but affect only poorly tissue levels of dopamine or norepinephrine.[20,21]

Dopamine β-Hydroxylase Inhibition

The final step in the biosynthesis of norepinephrine is accomplished by the hydroxylation of the β-carbon of dopamine by dopamine β-hydroxylase. An excellent review of the biochemistry of this system has been compiled recently,[22] and the reader is referred to it for details on this enzyme system. The authors have also commented on possible inhibitors, but conclude that to date no competitive inhibitor of dopamine β-hydroxylase which is effective *in vivo* is known. It should be emphasized that the substituted benzyloxyamines and benzylhydrazines as described earlier[23] were effective *in vitro*, but poorly or not effective in lowering endogenous norepinephrine levels.

At present the only β-hydroxylase inhibitors which are effective *in vivo* are the copper chelating agents, and disulfiram has received the greatest use for this purpose. Relatively large doses of disulfiram will lower tissue levels of norepinephrine but will raise dopamine.[24-26] Similar observations were made by Collins[27] who used the compound diethyldithiocarbamate, a metabolite of disulfiram. Bhagat and Gilliam[28] also demonstrated that disulfiram retarded the norepinephrine repletion in rat heart after depletion by tyramine pretreatment, suggesting that norepinephrine synthesis had been blocked. With the lowering of tissue norepinephrine due to inhibition of dopamine β-hydroxylase by disulfiram, the physiological response to nerve stimulation of organs innervated by adrenergic fibers is reduced concomitantly.[29]

Monoamine Oxidase Inhibition

In many organs innervated by adrenergic fibers the regulation of dopamine and norepinephrine levels is to a large extent the function of monoamine oxidase (MAO). This is apparent from the increase in amine levels resulting after blockade of MAO. The enzyme works through an oxidative deamination process and, in the presence of aldehyde dehydrogenase, forms the pharmacologically inactive carboxylic acid metabolite of the amine.

In spite of the declining therapeutic use of MAO inhibitors, interest in these compounds still prevails as evidenced by the numerous recent scientific publications. The picture of MAO is still far from complete, and the inhibitors are useful tools in its biochemical and physiological investigations as well as in determining the mechanism of other amine-related compounds, such as reserpine, α-MMT, and the indirect acting sympathomimetic amines.

Several important clinical contributions in this area during the past two years were (1) the reviews on the toxicity of the MAO inhibitors[30,31] in which an attempt was made to explain the direct adverse reactions as well as the interactions that occurred between the MAO inhibitors and various substances, and (2) a comprehensive review on the clinical pharmacology of tranylcypromine.[32] From these and previous articles it is quite apparent that all of the long acting MAO inhibitors have many actions, and most of these are related to the inhibition of MAO and the subsequent alteration of amine levels. This is probably true for the hypotensive action which is inherent with most of the long acting MAO inhibitors, including the newest addition, modaline sulfate (W3207B).[33] The mechanism of the blood pressure lowering effect of the MAO inhibitors has been attributed to various causes, but the most attractive hypothesis based on MAO inhibition is that of Kopin and associates.[34,35] These authors postulate that after MAO inhibition an accumulation of octopamine (β-hydroxytyramine) occurs at adrenergic nerve terminals because of the loss of the normal deamination pathway. The octopamine is thought to replace part of the normal stores of sympathetic mediator, and upon stimulation, it is released as a "false transmitter." Octopamine, however, is at best a poor substitute for norepinephrine, and this results in a diminished sympathetic response and hypotension. Other β-hydroxylated sympathomimetics have subsequently been found to behave in a similar manner to octopamine in replacing the normal sympathetic transmitter.[36] The "false transmitter" concept has received support from Farmer[37] who demonstrated the impairment of sympathetic nerve responses after administration of DOPA and dopamine in the presence of MAO inhibitor.

The literature abounds with descriptions of observations of the interactions of MAO inhibitors with different substances. Some of the interactions are difficult to interpret; this is especially the case with inhibitors having the phenethylamine or phenethylhydrazine structures. Such agents have the ability to (1) inhibit MAO; (2) cause increase in tissue levels of 5-HT, dopamine, norepinephrine, and probably amines which are normally absent, such as octopamine, tryamine, and tryptamine; (3) cause release of norepinephrine and dopamine because of its indirect sympathomimetic effect; and (4) produce other direct effects unrelated to MAO inhibition, such as an amphetamine-like central stimulation. It is no wonder then that phenelzine or tranylcypromine could enhance the toxicity of amphetamine, probably due to summation of effects of

the two drugs.[38] This also may be the case with the potentiation of the hyperthermic response of amphetamine, ephedrine and deoxyephedrine after MAO inhibition.[39] Inhibition of the enzyme cannot be the sole mechanism, for these sympathomimetic substances are not substrates of MAO. However, the interaction occurring between the MAO inhibitors and the antidepressants of the imipramine-like agents appears far more complex.[40]

The protection against depletion of catecholamines and/or 5-hydroxytryptamine (5-HT) by reserpine, tetrabenazine and guanethidine by the MAO inhibitors is well known, although the mechanisms of these interactions are still not clear. One explanation is that the excitation observed in mice given reserpine following a MAO inhibitor consists of two phases--the first attributable to the liberation and accumulation of catecholamines, and second, of 5-HT, in the active form in the central nervous system as a consequence of MAO inhibition.[41] Fielden and Green[42] also attributed the protection by iproniazid of the depletion of heart norepinephrine induced by guanethidine to the inhibition of MAO and not to a "bretylium-like" action of the inhibitor. In spite of these evidences it is still difficult to resolve whether the "protection" of amine depletion by reserpine or guanethidine is in fact protection from release, or protection from degradation of the released amine. The latter possibility would appear more reasonable, but work by Clementi[43] as well as earlier investigations which demonstrate protection by iproniazid of the morphological integrity of adrenal medullary granules from reserpine requires further clarification.

Other recent studies also point out the variation of complexities of the actions of MAO inhibitors, and these generally conclude that the anti-MAO activity itself is not always the primary mechanism for the effects that they observe.[44-48]

In discussing drug interactions with MAO inhibitors it should be pointed out that under certain conditions the action of the MAO inhibitor may be modified. Thus, the red blood cell and its constituents, heme and hemoglobin, are capable of decreasing the anti-MAO activity of several of the hydrazine-type inhibitors, but not the non-hydrazine compounds.[49] It was concluded that the hydrazines reacted with the heme portion of the red cell to become chemically inactivated. A similar observation was made by Vitek and Rysanek[50] who found that D-cycloserine blocked the actions of isoniazid but not pheniprazine and tranylcypromine. The authors concluded that isoniazid probably formed an inactive hydrazone with cycloserine. Antagonism of the irreversible inhibition of MAO may also be produced by prior exposure of the enzymes to reversible inhibitors. Horita[51] took advantage of this fact to produce a selective block of brain MAO by pretreating rats with BW 392C60 (a highly charged reversible inhibitor of MAO) and followed by pheniprazine. In so doing the peripheral MAO was protected from the long acting effects of pheniprazine, while brain MAO was inhibited without hindrance by the reversible inhibitor.

Considerable work on the nature of inhibitors of MAO activity still continues. Hustzi and Borsy[52] investigated the anti-MAO activity of diethyltryptamine and its analogues and found that differences in activity occurred. As with earlier authors they noted that the substrate used in the preparation also affected greatly the inhibitory activity. The variance of inhibitor activity with different substrates was also noted by Burger and Nara[53] who

employed various enzyme preparations, including pure beef plasma MAO. Their data are especially interesting because of the demonstration that the anti-MAO activity of several inhibitors varied so greatly depending upon the source and purity of the enzyme preparation. Fuller and Walters[54] described the inhibition of MAO by substrate amines and their α-methyl derivatives. No consistent relationship between degree of inhibition and efficacy as substrate could be found. Horita,[55] on the other hand, claims that certain of the arylalkylhydrazines, especially β-phenethylhydrazine (phenelzine) and γ-phenylpropylhydrazine, in addition to being irreversible inhibitors of MAO, may serve as substrates of this enzyme prior to exerting their inhibitor action. The evidence for this hypothesis is based around the loss of anti-MAO activity of phenelzine when exposed to preparations of MAO, and the prevention of this loss by prior treatment with other MAO inhibitors. Similar results were seen in the intact rat.

Catechol O-Methyl Transferase (COMT) Inhibition

Much less work has appeared on the inhibition of COMT. Perhaps this is the case because of the availability of fewer specific inhibitors of the enzyme. The importance of COMT in the metabolism of catecholamines is equally as great as for MAO, although the specific metabolic roles are quite different. COMT, which o-methylates the ring OH- groups of catecholamines, generally plays a greater role in the metabolism of endogenously administered or released catecholamines, while MAO is said to be more important for the intracellular regulation of the amines.

Recent investigations on the inhibitor activity of various compounds as possible COMT inhibitors include those of Ross and Haljasmaa.[56] Using the extract from mouse brain and tritiated norepinephrine as substrate they found the esters of gallic acid, D-catechin, 4-methylesculetin, 3,4-dihydroxymandelamide, 8-hydroxyquinoline and 4-methyltropolone were as active or greater than pyrogallol as inhibitors of COMT. The same authors also determined the *in vivo* efficacy of these compounds, but unlike the *in vitro* results, they observed that the compounds varied in potencies between brain and peripheral structures.[57] Furthermore, many of the compounds tested interfered with other adrenergic mechanisms, such as stimulation or blockade of adrenergic β-receptors. Pyrogallol appeared to be the most specific inhibitor for brain COMT *in vivo*, while the tropolones were most effective against COMT in peripheral structures. In intact animals, Burba and Murnaghan[58] found that demethylpapaverine potentiated the epinephrine-induced pressor responses and isoproterenol-induced depressor responses and correlated this to the inhibition of COMT which they had determined *in vitro*.

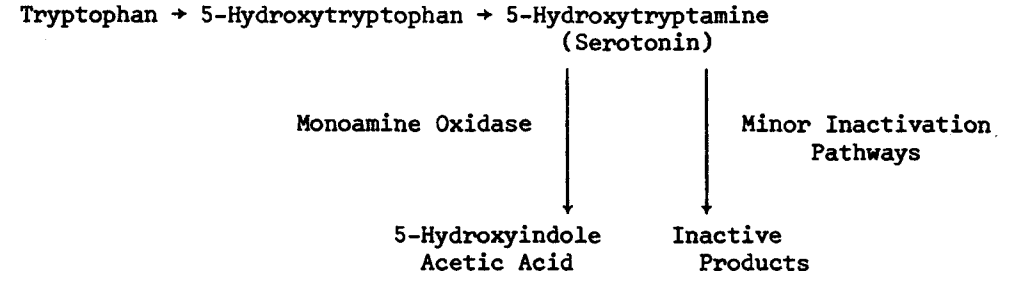
The COMT system should not be confused with the microsomal enzyme that o-methylates dihydroxy compounds.[59,60] A number of differences exists between the two systems, including distribution, species, pH optima, and substrate specificity.

INHIBITORS OF 5-HYDROXYTRYPTAMINE (SEROTONIN, 5-HT) METABOLISM

Although the height of popularity enjoyed by 5-HT in the 1950's as an area of pharmacological, biochemical and pathological research has subsided, much interest and investigative activity continue in an attempt to elucidate its

properties and possible biological functions. Several outstanding reference sources on the various aspects of 5-HT and other indoles are available.[61,62] The most recent comprehensive coverage on this subject is the monograph by Garattini and Valzelli[63] which includes references through 1964.

The metabolic pathway of 5-HT, like the catecholamines, involves the steps of hydroxylation and decarboxylation in its synthesis, while deamination plays the major role of degradation. Several other systems are also known, but these probably play a lesser part in the regulation of 5-HT metabolism. The following scheme represents the major steps in the formation and breakdown of 5-HT.



Currently, the enzyme involved in the first step of 5-HT biosynthesis, namely tryptophan hydroxylase, is undergoing experimental scrutiny. Its ability to transform tryptophan to 5-hydroxytryptophan (5-HTP) in intact animals was seen earlier, but until recently this enzyme could not be demonstrated *in vitro*. Tryptophan hydroxylase activity has now been reported to occur in dog brain,[64] rat liver,[65] and in neoplastic mast cells of the mouse.[66-68] It should be stressed that in the liver and neoplastic mast cell the enzyme may not represent the true tryptophan hydroxylase of brain tissue since the former also act as a phenylalanine hydroxylase. *In vivo* conversion of tryptophan by brain to eventually form 5-HT has been demonstrated.[69-72]

Inhibitors of tryptophan hydroxylase have been sought, but thus far highly active agents are not known. α-Methyl-DOPA, α, β, β-trimethyl-DOPA, and to some extent, α-propyl-3,4-dihydroxyphenylacetamide have been found to inhibit rat liver tryptophan hydroxylase.[73,74] Fuller[75] also surveyed a number of compounds as potential hydroxylase inhibitors, and many of the tyrosine and phenylalanine derivatives showed variable activities *in vitro*. 6,7-Dihydroxycoumarin demonstrated considerable *in vitro* activity, exerting some 60% inhibition at a concentration of 10^{-6}M.

5-Hydroxytryptophan decarboxylase, the enzyme responsible for the conversion of 5-HTP to 5-HT, is presumably identical with the aromatic amino acid decarboxylase discussed under the catecholamines. Inhibitors which were described under the aromatic amino acid (DOPA) decarboxylase would also apply here.

The degradation of 5-HT occurs primarily via monoamine oxidase, and this enzyme system and its inhibitors have been described earlier. 5-HT serves as a better substrate than norepinephrine; consequently, the inhibition of MAO in intact animals generally causes a more rapid and greater increase in tissue levels of 5-HT. This increase appears to occur within specific serotonergic neurons.[76]

INHIBITORS OF HISTAMINE METABOLISM

Like the other two biogenic amines discussed above, histamine biosynthesis involves the decarboxylation of its amino acid precursor, histidine. The enzyme, histadine decarboxylase, is not identical with the aromatic amino acid decarboxylase which converts both DOPA and 5-HTP to their corresponding amines. The breakdown of histamine is catalyzed by the enzymes diamine oxidase and imidazole N-methyl transferase. The former system oxidatively deaminates histamine, much like MAO on monoamines, while the latter enzyme inactivates histamine by methylating on the nitrogen at the 1-position. Upon formation of this intermediate it becomes susceptible to deamination via MAO to undergo further transformation.

With the resurgence of interest in the possible biological role of histamine as a regulator of the microcirculation and as a neurotransmitter, studies on the manipulation of histamine synthesis by inhibiting histadine decarboxylase are increasing. Actually there are at least two histidine decarboxylase enzymes present in mammalian tissues. Only the specific histidine decarboxylase is of concern here, for it appears to be the primary source of endogenous tissue histamine. Inhibition of this enzyme has been produced both *in vitro* and *in vivo* by D-2-hydrazino-3-[4-imidazolyl] propionic acid (hydrazino-histidine) and by 4-bromo-3-hydroxy benzyloxyamine (NSD-1055).[77] After administration of these compounds tissue levels of histamine decrease to varying degrees. Levine[78] also demonstrated the effectiveness of these compounds to reduce the basal secretion of acid in the rat stomach, presumably through a decreased formation of histamine. Thus, compounds which are effective inhibitors of specific histidine decarboxylase may aid in determining the biological functions of histamine.

In addition to inhibition, histidine decarboxylase also undergoes the unusual property of increased activity under certain conditions, including the influence of various pharmacological agents. Adrenergic agents such as epinephrine, pronethalol, DOPA and dopamine have been shown to increase histamine formation in mice, presumably through an increased activity of histidine decarboxylase.[79]

Diamine oxidase (DAO) has long been known to be a major degradation pathway for histamine and other diamines. Being a pyridoxal dependent enzyme earlier investigators employed carbonyl trapping agents, such as semicarbazide, isoniazid and aminoguanidine as inhibitors. The last named is still one of the most potent diamine oxidase inhibitors known. Other recent additions to the list of inhibitors (most of which are poorer than aminoguanidine) of diamine oxidase include mescaline and papaverine.[80]

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Section VI Topics in Chemistry

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Chap. 26 Synthetic Peptides

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Introduction.

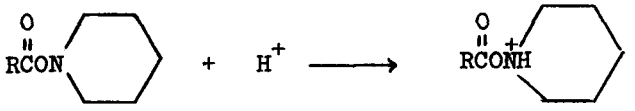
Emphasis in this short review will be placed on developments of methods of synthesis and their applications to the synthesis of biologically important peptides. Procedures are now good enough for the rapid synthesis of peptides containing up to a dozen amino acids, and with more difficulty, complex peptides such as insulin (51 amino acids). The development of "solid phase" synthesis and its automation by Merrifield (1) promises much. Meanwhile, the synthesis of dozens of analogs of such important peptides as oxytocin and bradykinin has already been accomplished, and structure-activity relationships are becoming clearer.

Reviews. Of particular importance is the book by Schröeder and Lübke (2), The Peptides, Vol. I. Methods of Peptide Synthesis which was published in 1965. This is a critical, well written, comprehensive survey, which covers the literature to nearly the end of 1964. The tables listing amino acid derivatives which are useful in synthesis are especially valuable.

A short review by the present author intended as a quick survey of the present state of the field of synthetic peptides also appeared in 1965(3).

Conference Report. The proceedings of the Seventh European Peptide Symposium, held in Budapest on September 3-8, 1964, have been published as a special volume of Acta Chimica Hungaria (4). The 38 research reports are indicative of the problems receiving attention in 1964, and to a large extent in 1965. Seven papers were concerned with new protecting groups or removal of protecting groups, six with racemization, two with aminoacyl incorporation into peptide chains, five with methods of forming the peptide bond, five with special problems and thirteen with the synthesis of naturally occurring peptides and analogs. Since more manpower was also involved in the latter, it is obvious that the actual synthesis of peptides is receiving the most effort. Along with this synthesis, of course, problems of methodology are met and solved. Some of these papers will be referred to in specific discussions to follow.

Methods of Forming the Peptide Bond. Among the more interesting active esters are the 1-hydroxypiperidine esters discovered by Young and associates (5). These are generally unreactive in peptide formation in neutral or basic conditions, but react readily in the presence of acetic acid. Activation by protonation is proposed:



As racemization, generally speaking, is a base catalyzed reaction, it was expected and experimentally verified that N-hydroxypiperidine esters of acylamino acids were not racemized by triethylamine. In contrast, other active esters such as p-nitrophenyl and N-hydroxysuccinimide esters are racemized by triethylamine, although they are stable under neutral or acidic conditions (6). Since the latter esters can be used for peptide formation without racemization, the principal virtue of the N-hydroxypiperidine esters would appear to be stability during their synthesis and storage. Unfortunately, Young and associates found that the direct synthesis of such esters of benzoyl-L-leucine, a test case analogous to what would be encountered in the synthesis of active esters of peptides, was accompanied by racemization when customary procedures were used. This is also true in the synthesis of other active esters.

Active esters of peptides can be made by starting with an amino acid active ester and building on the amino end, and then these can be used without racemization. However, until a simple method for the direct synthesis of active esters of acylpeptides without racemization is found, their practical use is restricted to derivatives of acylamino acids where the acyl group is benzyloxycarbonyl or the like. The good results with such esters makes their use quite popular today.

Further experience with N-hydroxysuccinimide (7), (30) and 2,4,5-trichlorophenyl(8) esters has been favorable, indicating that these derivatives will compete with p-nitrophenyl esters in popularity. A new class of reagents for making all three types of esters was reported by Sakakibara and Inukai (9); this consists of the trifluoroacetyl esters of the respective phenols or N-hydroxysuccinimide. These reagents are so reactive that attempted isolation brings about decomposition, but excellent results were obtained by the use of crude reagents. Furthermore, it was not necessary to isolate the active esters before reaction with amino acid esters to form dipeptides, which were obtained in excellent yields.

Continuation of the investigation of esters of p-phenylazophenol by Barth (10) has shown that they may be considered to be equivalent to p-nitrophenyl esters with the additional advantage that they are detectable by their color or UV fluorescence.

Among new active esters proposed in 1965 are those derived from 8-hydroxyquinoline (11), α-selenonaphthol (12), 3-hydroxypyridine (13), 3-nitroacetophenone (14) and 1-phenyl-3-methylpyrazolone-5 (14). The use of acylguanidines as intermediates in peptide synthesis (15) is reminiscent of esters of N-hydroxypiperidine in that they are activated by protonation. The scope of this method is yet to be determined.

Two investigations of 1-dialkylamino-1-alkynes (ynamines) (16), (17) have shown that racemization occurs if these reagents are used to activate acylpeptides, hence their use should be restricted to the activation of suitably protected amino acids.

Rapid Synthetic Procedures. The outstanding development to date is the "solid phase" method of Merrifield (1). The success of this procedure depends upon making the peptide insoluble by attachment at the carboxy end to a polymer, and adding acylamino acid units to the growing chain quantitatively. t.Butyloxycarbonylamino acids (BOC amino acids) in large excess along with

dicyclohexylcarbodiimide reagent are used, with subsequent removal of the BOC group by acid at each step. All by-products and excess reagents are removed by washing at each step. The procedure has been automated, with the addition of an amino acid unit to the growing peptide chain every four hours; bradykinin was synthesized in this fashion. By non-automatic procedures, a large number of bradykinin analogs were made (18), and some angiotensins (19).

A preliminary communication in 1963 by Letsinger and Kornet (20) reported a "solid phase" method of building from the carboxy end of a peptide chain using isobutyl chloroformate as the reagent. This has not been followed up, probably because conditions to avoid racemization were not known. Recent work in the writer's laboratory (21) has disclosed conditions which should be safe, hence this method can now be used.

An interesting variation of the Merrifield approach using a soluble polymer support and N-hydroxysuccinimide esters of BOC amino acids was reported in 1965 by Shemyakin and associates (22).

A new approach to rapid synthesis in aqueous solution using a water soluble carbodiimide as reagent and lengthening the peptide chain from the carboxylic end was reported by Knorre and Shubina at Budapest (23). Although ingenious, this method faces difficulties, the greatest being the possibility of racemization. Another procedure using a water soluble carbodiimide builds the peptide chain from the amino end (24). This method in its present form suffers from variable yields and the number of manipulative procedures.

One can see a definite advantage of an automatic machine for making small amounts of peptides, but there may be no advantage if large quantities are desired. The development of a safe general procedure (other than the azide method, which has disadvantages) of combining peptides without racemization would diminish the importance of the one-at-a-time process for moderate-sized or large peptides. Further improvements of rapid conventional procedures which give good yields might also nullify some of the advantages of the machine approach. In the author's experience, the mixed carboxylic-carbonic anhydride procedure (21) offers excellent possibilities for such developments.

Amine-Protecting Groups. The ease of removal of the benzyloxycarbonyl group by hydrogen bromide in acetic acid and the increasing recognition of the dangers of handling peptides under alkaline conditions have focussed attention in recent years on protecting groups which can be removed by mild acidic conditions. Of a number of amine-protecting groups of this type, the most widely used is the t-butyloxycarbonyl group (25). Favorable results with t-amyloxycarbonyl (26), benzhydryloxycarbonyl (27), p-methoxybenzyloxycarbonyl (28), and o-nitrophenylsulfenyl (29) groups reported during the year shows that these may have comparable use. Cleavage of the o-nitrophenyl group by Raney nickel is of interest (30).

A preliminary report on use of the acetoacetyl group for amine protection (31) is noted. Electrolytic reductive splitting of benzoyl and tosyl groups from amino acids and peptides has been accomplished in the presence of tetramethylammonium chloride (32).

Carboxy Protecting Groups. Benzhydryl esters of several acylamino acids have been synthesized by the azeotropic distillation method or by the reaction of the acid with diphenyldiazomethane (27), (33). They are comparable to t-butyl esters in acid lability.

Selective reactivity of an activated acylamino acid at the amino group of glycylhydrazide or glycylglycylhydrazide was demonstrated, hence a hydrazide might be useful as a protecting group (34).

Special Protecting Groups. In contrast to S-diphenylmethylcysteine, S-4,4'-dimethoxydiphenylmethylcysteine gives a color on thin-layer chromatograms upon treatment with hydrogen chloride, which should be a useful property (35).

A useful, comprehensive survey of the protection of the guanidino group of arginine was presented at Budapest by Guttmann and Pless (36). New groups proposed for this function are p-nitrobenzyloxycarbonyl, which is resistant to acidolysis and easily cleaved by catalytic hydrogenation, and 3,4,5,6-tetrachloro-2-isopropylphthalyl (TIP), which is resistant to acidolysis and hydrogenation but readily removed by aqueous acid. Bajusz (37) reported good results with the t-butyloxycarbonyl (BOC) group. When the nitro group is used, it may subsequently be removed by electrolytic reduction (38).

Conversion of Serine-Containing Peptides to Cysteine-Containing Peptides. The conversion of O-tosyl-L-serine peptides to S-acetyl or S-benzoyl-L-cysteine peptides by thioacetate or thiobenzoate ions has been studied in detail (39). This simple procedure promises to be particularly useful in enzyme studies.

Racemization Studies. The combination of acylpeptides with amino acid or peptide derivatives without racemization of the carbon alpha to the carboxylic group (starred in equation below) continues to present problems.

$$\text{Acyl}\cdot\text{NH}\overset{*}{\underset{R}{\text{C}}}\text{HCOOH} + \text{H}_2\text{N}\underset{R'}{\text{C}}\text{HCOOR}'' \longrightarrow \text{Acyl}\cdot\text{NH}\overset{*}{\underset{R}{\text{C}}}\text{HCONH}\underset{R'}{\text{C}}\text{HCOOR}''$$

In this case, the acyl stands for an acylamino acid or acylpeptide. If R is H or part of the prolyl residue, there is no danger, and hence many synthetic schemes involve coupling of peptides when glycine or proline are C-terminal. Otherwise, the safest procedure is activation through an azide, and this often gives unsatisfactory results. Other activation procedures can often be used with proper control of conditions, but the uncertainties make these unpopular. Hence the building of peptide chains by the addition of acylamino acids to the amino end of a peptide chain has become the most popular procedure today. This procedure has been successful, perhaps as best exemplified by Merrifield's "solid phase" procedure. However, considering the flexibility and yield advantages of safe procedures for the combination of peptides, it is surprising that very little basic work to solve the problem was reported during 1965. It is the author's opinion that some of our common activation methods will give satisfactory results when properly used, and more basic research with them is needed to determine the safe conditions. Promising results for the mixed carbonic anhydride method have recently been reported (21).

G. T. Young and associates have continued their research on racemization mechanisms, with proof that racemization of benzyloxycarbonylglycyl-L-phenylalanine p-nitrophenyl ester by triethylamine proceeds through an oxazolone mechanism (40). From previous work from the laboratories of Young and of others, it seems likely that this is the common mechanism of racemization of activated acylpeptides during peptide synthesis.

B. Liberek has also continued his studies on racemization of amino acid derivatives where direct abstraction of the α hydrogen is the favored mechanism (41). Taschner and associates have shown that cyanomethyl esters made with bromoacetonitrile are less likely to be racemized than those made with chloroacetonitrile (42).

Basic information on racemization in several methods of peptide synthesis was reported by Anderson, Callahan and Zimmerman (43). Further studies by Beyerman, Weygand and associates with 1,2,4-triazole as a catalyst for the reaction of several active esters in peptide synthesis have given no indication of racemization, whereas imidazole, as expected, can cause racemization (44).

Schnabel (45) has shown that azlactones from formyl- and trifluoroacetylamino acids and from benzyloxycarbonyldipeptides are readily made by treatment with dicyclohexylcarbodiimide. Most of these are at least partially optically active, and reaction with ethyl glycinate results in more or less strong racemization which is diminished in the presence of acetic acid. Indirect evidence was obtained that symmetrical anhydrides of benzyloxycarbonylamino acids are formed by the reagent, and these are racemized partially during synthesis and aminolysis. The conclusions drawn about racemization of the symmetrical anhydride from benzyloxycarbonylglycyl-L-phenylanine are also based on indirect evidence, and they need to be verified by experiments with isolated and characterized anhydride.

In agreement with observations that benzyloxycarbonylamino acids are not normally racemized by dicyclohexylcarbodiimide, Vajda and associates (46) conclude that no azlactones are formed. Acylureas and diketopiperazines were detected, and these might have been derived from symmetrical anhydrides.

Synthesis of Biologically Important Peptides. This is the major goal of peptide synthesis, hence it is not surprising that the most effort has been spent in this field. It is not possible in this short review to refer to all the papers in this field which were published in 1965. Analogs of oxytocin, vasopressin, angiotensin eledoisin, bradykinin and kallidin continued to receive much attention. In a discussion of the possible mechanisms of action of peptide hormones, Boissonnas (47) estimates that the total number of analogs of vasopressin plus oxytocin which have been synthesized up to the end of 1965 at about 130, of αMSH plus ACTH at 25, angiotensin 50, bradykinin 60 and eledoisin 120.

In the antibiotic field, the syntheses of valine- and isoleucine-gramicidin A(48), polymyxin B_1(49), and Colistin-A(50) should be noted.

In the enzyme field, the investigations of Hofmann and associates (51) on the synthesis of peptides related to the N-terminus of bovine pancreatic ribonuclease deserve special comment. Formation of the peptide bond under aqueous conditions (organic solvent plus water) was frequently accomplished with good results, using active ester or azide intermediates and free peptides. With peptides of increasing size and water solubility, one can anticipate that the method will become correspondingly important. The last paper in the series, which discusses structure-activity relationships, well illustrates the importance of synthetic peptides for the development of understanding of biological mechanisms.

Before 1965, the synthesis of insulin peptides with biological activity was reported from three laboratories; further progress from all three has recently been reported. In brief communications (52), Katsoyannis and associates have reported the combination of synthetic A and B chains of human insulin with a 2% yield of insulin activity, and hybrids of synthetic human A and natural bovine B (4 to 8%) and synthetic human B with natural bovine B (8%). Kung and associates (53) reported the isolation of crystalline synthetic bovine insulin with activity greater than 20 I.U./mg. (natural insulin approximates 24 I.U./mg.). Zahn and associates (54) found that pre-oxidation of the A chain improved the yield of insulin upon oxidative recombination of the A and B chains. Work from the three laboratories indicates that proper conditions for recombination of the A and B chains are the most important factor for high activity yields. Since the formation of three disulfide bonds (one intra-chain, two inter-chain) is involved, this is understandable. A number of detailed synthetic papers from the three groups will not be referred to here. A paper concerning preparative and analytical problems incurred by Zahn's group (55) is recommended as an indication of the scope of the problem of insulin synthesis. Insulin, with 51 amino acids, is the largest peptide (or small protein) synthetized to date.

Indicative of new approaches constantly being developed is the synthesis of an insulin fragment by Zervas et al. (56). Here, by selective use of S- and N- protecting groups, a peptide containing the 6-12 amino acid fragment of the A chain of sheep insulin, with the intra disulfide link, was synthesized. A complete A chain with this link would make combination with the B chain simpler, and would likely accomplish in a more elegant way the result of the A chain pre-oxidation step of Zahn.

D-Amino Acid Analogs. Although some peptide antibiotics contain D amino acids, peptide hormones do not. Before 1965, it had been shown with several hormones that the replacement of one L-amino acid with the D-isomer usually lowered activity but did not destroy it. It remained to be seen what the effect of complete replacement would be. During the year, results were reported for several hormones. With D-bradykinin (57), D-oxytocin (58), and D-Val^5-Angiotensin II-Asp^1-β-Amide (59) no hormone activity or inhibition of the activities of the L-analogs were found. Partial replacement of L-amino acid by D-amino acid residues in eledoisin lowered activities to a variable degree (60). The melanocyte-stimulating activity of a pentapeptide fragment of α-melamocyte-stimulating hormone was, in contrast, inhibited by the all-D analog (61). With the C-terminal tetrapeptide derivative of the hormone gastrin, BOC·try-met-asp-phe·NH_2, activity was lost by partial or complete replacement of the L-amino acids by the corresponding D-amino acids, and inhibitory activity was not found (62).

Cyclic Peptides. The principal interest here also is the synthesis of naturally occurring peptides or analogs, and several such syntheses were reported. The p-nitrophenyl ester method was favored for the cyclization step, although other coupling procedures were also used. Syntheses of polymyxin B_1 (49), colistin-A (50), evolidine(63) and an active analog of gramicidin S(64) were reported.

An interesting technique involving ester attachment of an amino acid to an insoluble poly(nitrophenol), building a peptide chain to this by stepwise addition at the amino end, and finally intra-molecular cyclization was disclosed (65).

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Chap. 27 NUCLEOSIDES AND NUCLEOTIDES

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Over the past few years, several excellent reviews (1,2) and books (3,4) have appeared on nucleosides and nucleotides. During 1965 considerable interest in the nucleosides and nucleotides has been maintained as evidenced by the significant research activity in this area. Because brevity has been requested in this report, some of the papers published during 1965 could not be included.

The preparation of nucleosides by the fusion (5,6,7) of purines with fully O-acylated sugars in the presence of an acid catalyst is being used extensively because of several advantages: (a) it avoids the necessity of preparing a heavy metal salt of the purine and thereby insures that the nucleoside will not be contaminated with a metal which could interfere with studies on biological activity, (b) it obviates the necessity of preparing a chloro sugar, and (c) the yield of the nucleoside is frequently higher than in the conventional synthesis. It has been reported (8) that bis-(p-nitrophenyl)hydrogen phosphate is an effective catalyst for the synthesis of purine nucleoside by the fusion method. For example, the fusion of theophyline with penta-O-acetyl-β-D-glucopyranose in the presence of bis-(p-nitrophenyl)hydrogen phosphate gave a 47% yield of the 7-substituted nucleoside. A synthesis of 4-oxo-3-ribofuranosyl dihydropyrimidine by the fusion of 4-hydroxypyrimidine and a tetraacylribose derivative was described (9). Condensation of purines, benzimidazole, or N^6-benzoylcytosine and acylglycosyl halides in nitromethane in the presence of mercuric cyanide gave the corresponding nucleosides in higher yields than in the conventional method (10). A variety of nucleosides were prepared by the condensation of the bis-(trimethylsilyl)ether of 5-substituted (H,CH_3,F,Cl,Br,I and -$CH_2OCH_2C_6H_5$)uracils with several glycosyl halides(11).

The utility of the directive influence of a removable blocking group at N-3 on an adenine nucleus has been demonstrated by the synthesis of 7α-D-ribofuranosyladenine, which was shown to be identical to the nucleoside from pseudovitamin B_{12}. The synthesis of this nucleoside was accomplished by the condensation of the mercuri salt of N_6-benzoyl-3-benzyladenine with 2,3,5-tri-O-acetyl-D-ribofuranosyl chloride followed by deacylation and removal of the 3-benzyl group by catalytic hydrogenolysis (12). Other investigators who studied the product distribution in the ribosylation of mercuri salts of certain purines and deazapurines found that the ratio of the 9-substituted to 7-substituted isomer was usually greater than one for those purines which contained a nitrogen at position 3 (13). In the benzimidazoles, the ratio of isomeric products was closer to unity (13). However, certain exceptions

were noted, and it is apparent that steric effects, as well as electronic effects are important in determining the position of reaction in purines and deazapurines.

Ikehara and Tada have prepared an 8,2'-cyclonucleoside by allowing 2-chloro-8-mercapto-9-(2,5-diacetyl-3-tosyl-β-D-xylofuranosyl)adenine to react with sodium methoxide. Raney nickel desulfurization of the 8,2'-cyclonucleoside, followed by hydrogenolysis of the 2-chlorine atom with a palladium catalyst led to 2'-deoxyadenosine (14). The synthesis of 9-β-D-ribofuranosyl uric acid was accomplished by converting 8-bromoguanosine into 8-benzyloxyguanosine, which on treatment with nitrous acid followed by catalytic hydrogenolysis of the benzyl group gave the desired product (15). In addition, 8-aminoadenosine was synthesized from 8-bromoadenosine (15).

Robins and Robins (16) have employed the fusion method for the synthesis of 2'-deoxy-9-α-and β-ribofuranosylpurine. Thus, fusion of 6-chloropurine and 1,3,5-tri-O-acetyl-2-deoxy-D-ribofuranose in the presence of chloroacetic acid gave, after deblocking, 6-chloro-9-α-and 9-β-D-ribofuranosylpurines. Similar studies were carried out with 2,6-dichloropurine, 2,6,8-trichloropurine, N^6-acetyladenine, benzimidazole, 5,6-dimethyl-,5,6-dimethoxy-, and 5,6-dichlorobenzimidazoles (16,17). An NMR study of the 2'-deoxyribofuranosylpurines and benzimidazoles revealed that the β-anomer exhibits a pseudo-triplet with $J_{H'}$= 7 c.p.s. and a peak width = 14 c.p.s. for the anomeric proton whereas the α-anomer exhibits a multiplet of four with $J_{H'}$ = 3 and 7 c.p.s. and a peak width = 10 c.p.s. for the anomeric proton (16,17). The method of anomeric assignment by NMR for the 2'-deoxy-D-ribofuranosyl nucleoside appears to be general and is not dependent on the heterocyclic base (16).

The synthesis of 9-(3-deoxy-β-D-galactofuranosyl)adenine was accomplished by condensation of 1,2-di-O-acetyl-5,6-di-O-benzoyl-3-deoxy-D-galactofuranose with chloromercuri-6-benzamidopurine in the presence of titanium tetrachloride (18). In addition, 1,2-isopropylidene-3-deoxy-D-galactofuranose was reacted with periodic acid and then with sodium borohydride to give a 3-deoxy-L-arabinose derivative. Condensation of the appropriately blocked arabinose derivative with chloromercuri-6-benzamidopurine in the presence of titanium tetrachloride gave 9-(3-deoxy-α-L-arabinofuranosyl)adenine (18). In a related sequence of reactions, 1,2-di-O-acetyl-5,6-di-O-benzoyl-3-deoxy-D-glucofuranose on condensation with chloromercuri-6-benzamidopurine using titanium tetrachloride gave 9-(3-deoxy-β-D-glucofuranosyl)adenine (19). 1,2-Isopropylidene-3-deoxy-D-glucofuranose was converted into 1,2-di-O-acetyl-5-O-benzoyl-3-deoxy-D-ribofuranose which on condensation with chloromercuri-6-benzamidopurine and titanium tetrachloride gave 9-(3-deoxy-β-D-ribofuranosyl)adenine (19). 1,2-Isopropylidene-3-deoxy-D-glucofuranose was converted into 1,2-di-O-acetyl-5-O-benzoyl-3-deoxy-D-ribofuranose which on condensation with chloromercuri-6-benzamidopurine and titanium tetrachloride gave 9-(3-deoxy-β-D-ribofuranosyl)adenine (19). A different synthesis of some 3'-deoxyribofuranosyl nucleoside was reported and involved the condensation of 3-deoxy-2,5-di-O-benzoyl-ribofuranosyl bromide with chloromercuri-6-benzamidopurine (20). In

addition,the bromo sugar was also condensed with chloromercuri-2,6-dibenzamidopurine (20)with chloromercuri-6-chloropurine (20) and with chloromercuri-N^2-acetylguanine (21). From the 6-chloropurine nucleoside, a variety of 6-substituted derivatives were prepared (20), and it was found that 9-(3-deoxy-β-D-ribofuranosyl)-6-methylaminopurine was as potent an inhibitor of uridine incorporation into RNA in KB cells as was 9-(3-deoxy-β-D-ribofuranosyl)adenine (22).

Two groups (23,24) have described the synthesis of 9-(5-deoxy-β-D-xylofuranosyl)adenine. With the view of converting the xylonucleoside into the arabinonucleoside, a study of the monotosylation was undertaken (23). It was found that the reaction of 9-(5-deoxy-β-D-xylofuranosyl)adenine with p-toluenesulfonyl chloride in pyridine gave the 3'-O-tosylate. The formation of a 3'-O-tosylate rather than the expected 2'-O-tosylate was rationalized on the basis of hydrogen bonding between the 3'-hydroxyl and the N-3 nitrogen of the adenine nucleus which could make the 3'-hydroxyl more reactive. Consequently, it was reasoned (23) that monotosylation of the dianion of 9-(5-deoxy-β-D-xylofuranosyl)adenine should produce the 2'-O-tosylate, and under these conditions should give 9-(2,3-anhydro-5-deoxy-β-D-lyxofuranosyl)adenine. These results were obtained and on opening the epoxide with sodium benzoate in DMF, the desired 9-(5-deoxy-β-D-arabinofuranosyl)adenine was obtained along with smaller amounts of the corresponding xylonucleoside (23).

Two groups of investigators (25,26) have synthesized 5-trifluoromethyl-6-aza-2'-deoxyuridine. Montgomery and Hewson have devised an improved procedure for the preparation of 5-allyl-2'-deoxyuridine which involves the fusion of the bis-(trimethylsilyl)ether of 5-allyluracil with 3,5-di-(p-chlorobenzoyl)2-deoxy-D-ribofuranosyl chloride (27). The synthesis of 2',5'-dideoxycytidine was accomplished by the following sequence of reactions: N-benzoyl-2'-deoxycytidine was converted into its 5'-tosylate which was acetylated to give the completely blocked material. Reaction with iodide followed by deblocking with methanolic ammonia gave the 5'-iodo derivative which on catalytic hydrogenolysis gave 2',5'-dideoxycytidine (28).

A new synthesis of 1-β-D-arabinofuranosylpyrimidine has been described (29) which involves the condensation of 2,4-dimethoxypyrimidine or 2,4-dimethoxy-5-trifluoromethylpyrimidine with 2,3,5-tri-O-benzyl-D-arabinofuranosyl chloride. As reported by Glandemans and Fletcher, who originally used 2,3,5-tri-O-benzyl-D-arabinofuranosyl chloride for the preparation of 9-β-D-arabinofuranosyladenine (30), the formation of a "cis-nucleoside" with this blocked chloro sugar is probably caused by a Walden inversion at C-1. (29,30).

In an elegant series of papers, Doerr, Codington and Fox describe their work on the 2',5'- and 3',5'-epoxides of pentanofuranosyluracils (31,32). Thus, when 1-(5'-O-mesyl-2'-3'-epoxy-β-D-lyxosyl)uracil is allowed to react with sodium benzylate 1-(2',5'epoxy-3'-O-benzyl-β-D-arabinosyl)uracil is formed. Catalytic hydrogenolysis of the benzyl group produced 1-(2',5'-epoxy-β-D-arabinofuranosyl)uracil which was also prepared by the treatment of 1-(5'-O-mesyl-β-D-arabinofuranosyl)-

uracil with aqueous alkali. The synthesis of 1-(3',5'-epoxy-β-D-xylofuranosyl)uracil was accomplished by allowing 1-(5'-O-mesyl-β-D-xylofuranosyl)uracil to react with aqueous alkali (31). The synthesis of the 1-(2',5'-and 3',5'-epoxy-β-D-lyxofuranosyl)uracils was also described (32). Base treatment of 1-(5'-O-mesyl-β-D-lyxofuranosyl)-uracil gave 1-(2',5'-epoxy-β-D-lyxofuranosyl)uracil. The proof of structure of this compound(2',5'-epoxy derivative) was established by synthesis of the other isomer. Thus, 1-(3',5'-epoxy-β-D-xylofuranosyl)-uracil was converted into its 2'-O-mesylate which on treatment with sodium t-butoxide gave 2,2'-anhydro-1-(3',5'-β-D-lyxosyl)uracil. Aqueous base cleaved the 2,2'-anhydro bond resulting in the formation of 1-(3',5'-epoxy-β-D-lyxofuranosyl)uracil which on hydrolysis with aqueous acid gave 1-β-D-lyxofuranosyluracil (32). Recently, a facile synthesis of 2,2'-anhydroarabinopyrimidine nucleoside has been described by the reaction of 5'-O-trityluridine with thiocarbonyldiimidazole (33).

Because of an interest in the synthesis of the antibiotic amicetin, it was necessary to explore the general area of disaccharide nucleoside synthesis (34). The 1-halo sugar derivatives of heptaacetyl lactose and cellobiose were converted to their corresponding cytosine nucleoside derivatives by two established procedures. The β-D-configuration of the nucleoside bond was established by degradation to a known compound. In addition, an amino sugar disaccharide nucleoside by the condensation of 1-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-4-ethoxy-2(1H)-pyrimidone and 2-deoxy-2-benzamido-3,4,6-tri-O-acetyl-α-D-glucopyranosyl bromide (34).

Several reports have appeared in the literature on the synthesis of pyrimidine nucleosides derived from 2-deoxyhexoses (35,36). The synthesis of 9-(β-D-glucuronosylamide)adenine has also been described (37). The utilization of the 2,4-dinitrophenyl group as a blocking group for the synthesis of 2'-amino-2'-deoxynucleoside has been described, and it appears that this blocking group offers utility when it is desired to prepare the anomeric nucleosides since it shows no tendency to participate at C-1 (38). For example, the condensation of 3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino-α-D-glucopyranosyl bromide with chloromercuri-6-acetamidopurine gave, after deblocking, the α- and β-anomers of 9-(2-amino-2-deoxy-D-glucopyranosyl)adenines (38). The use of the benzylsulfonyl group as a nitrogen blocking group in the preparation of amino sugar nucleoside synthesis was demonstrated by the preparation 9-(2-acetamido-2-deoxy-β-D-glucopyranosyl)adenine from tri-O-acetyl-2-benzylsulfonylamino-2-deoxy-α-D-glucopyranosyl chloride and chloromercuri-6-benzamidopurine (39).

Several studies have appeared on the conversion of ribonucleosides into 3-amino-3-deoxyhexosyl nucleosides (40,41,42,43). Periodate oxidation of uridine followed by condensation with nitromethane gave a mixture of isomers from which the isomer with the gluco-configuration was obtained in approximately 60% yield. Hydrogenation of the nitro group with Raney nickel gave 1-(3-amino-3-deoxy-β-D-glucopyranosyl)-uracil (40) and when the hydrogenation was carried out with a platinum catalyst, the corresponding 5,6-dihydro derivative was obtained (40,41). In related studies (42,43), adenosine was oxidized with periodate and

the dialdehyde on condensation with nitromethane gave a mixture of isomers. Hydrogenation of the nitro group of these isomers allowed the separation of 9-(3-amino-3-deoxyhexopyranosyl)adenines of the gluco-, galacto- and manno- configurations (42). Finally, Brown and Read have shown that the product obtained by treating periodate-oxidized adenosine-5'phosphate with methylamine and then with sodium borohydride is 2-(9-adenyl)-4-methyl-6-phosphoryloxymethylmorpholine (44).

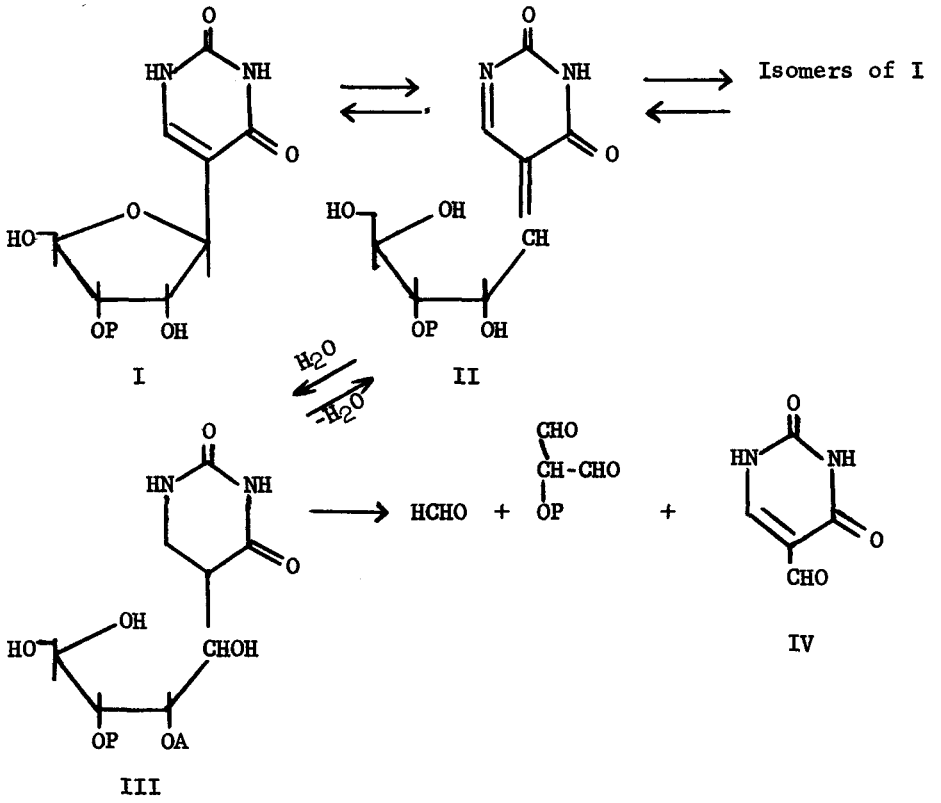
Broom and Robins have reported that the reaction of diazomethane and adenosine in a homogeneous solution of water and 1,2-dimethoxyethane produced 2'-O-methyladenosine (45). This selective reaction of the 2'-hydroxyl group of adenosine may find extensive application to the synthesis of polynucleotides if a functional group can be introduced, such as benzyl, which can be easily removed at a later stage.

In order to study the position of attachment of the aminoacyl group to the terminal residue in aminoacyl-S-RNA, Reese and Trentham have studied acyl migrations in ribonucleosides (46,47). The preparation of 3',5'-di-O-acetyluridines and, for the first time, 2',5'-di-O-acyluridine was reported, and a method (46) was developed to determine the position (2' or 3') of an acyl group which takes advantage of the fact that anhydronucleoside formation occurs more readily with a 2'-O-mesyl-3'-O-acyl derivative than a 3'-O-mesyl-2'-O-acyl derivative of uridine. Thus far, the mesylation analysis procedure is useful for 2'- or 3'-derivatives of uridine. A study of the equilibration of 2',5'-di-O-acyluridines and 3',5'-di-O-acyluridine was undertaken, and it was found that the 3',5'-diesters were only marginally more stable than the 2',5'-isomers. Thus, it was found by an acetylation study as well as an equilibration experiment that the ratio of the 3',5'-diester to 2',5'-diester was approximately 1.6 to 1 (47). The synthesis of some 2'(3')-O-aminoacyl derivatives of ribonucleosides and ribonucleoside-5'phosphate by the use of ortho ester derivatives of amino acids has been described(48).

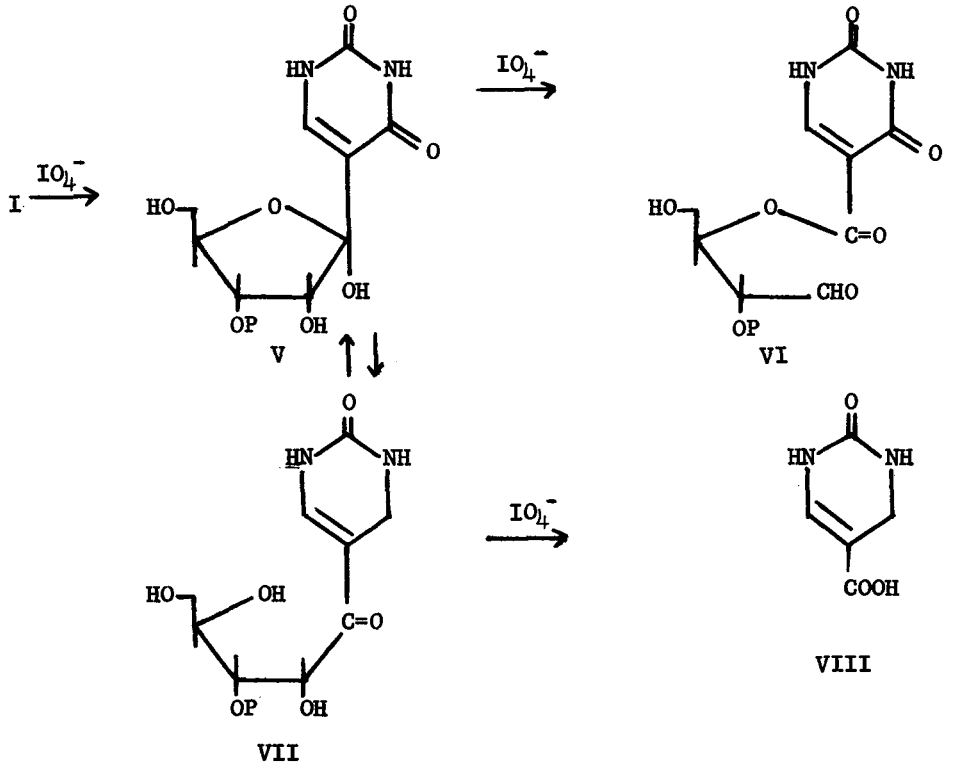
A variety of new 2',3'-ketal derivatives of ribonucleoside were synthesized with a view to extending the types of blocking groups for those reactions which convert ribonucleosides to 5'-substituted derivatives (49). A study of the rates of hydrolysis of the 2',3'-ketals of uridine at pH 2 and at 26° revealed that the half-life of the reaction varied from 0.5 hour for the crotonylidene derivative to more than 40 hours for the diphenylmethylidene derivatives. The other ketals studied with half-life shown in parentheses were: cyclooctylidene(2.5), cycloheptylidene (3), cyclopentylidene (4), methyl-t-butylmethylidene (20), isopropylidene (20) and diethylmethylidene (40). The methoxymethylidene derivative was also studied (49) and was found to have a half-life of 0.08 hour for the formation of a mixture of 2'- and 3'-O-formyluridine. Hampton has suggested that a 5'-O-alkoxyalkyl derivative of a nucleoside may have utility as a 5'-hydroxy blocking group because of its lability under acid or even neutral conditions (50). In the case of uridine the 5'-O-alkyoxyalkyl derivative is selectively prepared by an exchange reaction between the nucleoside and 2,2-dimethoxypropane (50).

In an interesting paper on the periodate oxidation of pseudouridine-

3'-phosphate (I) at pH 8.9, it was found that the products of oxidation were 5-formyluracil, 5-carboxyuracil, inorganic phosphate, formaldehyde, and unidentified products. As the pH is lowered toward 7, the oxidative reaction is slower, and 5-carboxyuracil appears to be the only heterocyclic product formed (51). Based on the observation tht pseudouridine and pseudouridine-2'(3')-phosphate is isomerized in either acid or alkali to give a mixture of two anomeric furanose derivatives and two anomeric pyranose derivatives, the following mechanism of periodate oxidation of I was proposed (51):



a priori, One might expect that the 5-carboxyuracil formed from I might arise by "overoxidation"of IV. However, when IV was exposed to potassium periodate, the rate of oxidation of IV to the 5-carboxy derivative was too low to account for the amount of 5-carboxyuracil from I. Therefore, a second pathway was proposed to account for the formation of VIII and involves the direct oxidative hydroxylation at C-1' to give V which by one of the pathways shown can give 5-carboxyuracil. Similar experiments were performed on pseudouridine-3',5'-phosphate (51).



Dekker has described a unique method for the separation of nucleoside mixtures on Dowex-1 (OH) using methanol and water mixtures. This separation procedure is based primarily on ion-exchange and consequently, should find wide application both for micro- as well as large-scale syntheses (52).

In an interesting report by Leonard and Laursen, the preparation of 3-β-D-ribofuranosyladenine (3-isoadenosine) and the corresponding 5'-phosphate was described (53). Because 3-isoadenosine supported the growth of an adenine-requiring Escherichia coli mutant and inhibited the growth of certain mammalian cell cultures, coenzyme analogs of 3-isoadenosine were prepared (54). 3-Isoadenosine-5-triphosphate replaced ATP in a hexokinase enzyme system and in the production of light in the luciferin-luciferase system. 3-Isoadenosine-5'-phosphate was converted in into the 3-isoadenosine analog of nicotinamide-adenine dinucleotide (NMN-3-iso-AMP) and this isomer was studied in a variety of dehydrogenase enzymes. Surprisingly, alcohol and glutamic dehydrogenase NMN-3-iso-AMP was reduced more rapidly than NAD (54). The observation that the 3-isoadenosine derivatives are active in enzyme systems will probably lead to a better understanding of the reaction mechanism and binding sites of certain enzymes.

Khorona and his coworkers have continued their extensive investigation on polynucleotides (55,56,57,58,59). For the synthesis of ribodinucleotides bearing a 3'-phosphomonoester group, a method was developed which involves the condensation of a fully protected purine or pyrimidine 3'-phosphate with uridine-2',3'-cyclic phosphate. This general approach should be applicable to all bases (55). Some (3'→5') linked diribonucleoside phosphates containing 3- and 5-methyluracil have, in general, been synthesized by the condensation of a 3'-nucleotide blocked at the 2'-and 5'-positions by trityl groups with a 2',3'-isopropylidene derivative of a second nucleoside (60). In addition, a general procedure for the isolation of "minor" nucleoside from the hydrolysates of ribonucleic acid has been described (61). The synthesis of dinucleoside phosphates by the "Anhydronucleoside Method" (62,63,64,65) is finding extensive utilization. For example, the synthesis of cytidylyl-(3'-5')-adenosine was readily accomplished by the condensation of 1(2,3'-anhydro-2',5'-di-O-trityl-β-D-xylorufanosyl)cytosine with adenylyl-5' benzoic anhydride (66).

The dismutation of adenosine 5'- triphosphate as its tributylamine salt in anhydrous pyridine has been found to produce two series of compounds: a homologous series of adenosine 5'-polyphosphates containing up to seven phosphate groups and a second minor series of α,ω-di(adenosine-5')polyphosphates. The presence of an excess of ortho or pyrophosphate leads to the formation of AMP and ADP (67). A study of the reaction mechanism of the dismutation of ATP to adenosine 5'-polyphosphates utilizing α-^{32}P-labeled-ATP and γ-^{32}P-labeled-ATP in pyridine led to the conclusion that pyridine attacks the γ-phosphorus of ATP to give ADP and a covalently bonded N-phosphorylpyridinium ion which is the reactive phosphorylating agent (68).

α-Adenosine-5'-phosphate has been synthesized by the condensation of 5-diphenylphosphoryl-D-ribofuranosyl bromide 2',3'-cyclic carbonate with chloromercuri-6-benzamidopurine and it was shown that α-adenosine-5'-phosphate is not a substrate for snake venom 5'-nucleotidase (69). For a program on the synthesis of phosphonic acid analogs of nucleoside phosphates, the preparation of 5'-adenylyl methylphosphonate and 5'-adenylyl chloromethylphosphonate was accomplished by the condensation of adenosine 5'-phosphate and the alkylphosphonic acid in the presence of dicyclohexylcarbodiimide (70). The synthesis of adenosine-5'-methylenediphosphonate was accomplished by the condensation of 2',3'-isopropylideneadenosine with methylenediphosphonic acid (71).

Baker, Tanna and Jackson have attempted to simulate 5'-phosphoribosyl binding to thymidylate synthetase and succinoadenylate kinosynthetase by the utilization of 5'-O-carbamates of nucleosides but the 5'-O-carbamoyl derivatives of 6-mercaptopurine ribonucleoside, thymidine, and 2'-deoxy-5-fluorouridine were noninhibitory against these two enzymes (72).

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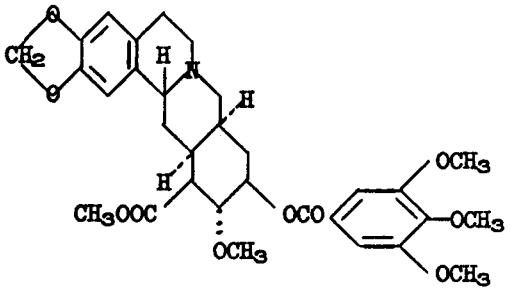
Chap.28 Alkaloids

William I. Taylor
CIBA Pharmaceutical Company

Work on synthetic reserpine derivatives continues, new 18-O-phenoxyacetic acid and phenylmercaptoacetic acid esters of methyl reserpate have been prepared[1] and were found to be much more weakly active than reserpine. Racemic 17-desmethoxydeserpidine synthesized by the Woodward route, in contrast to an earlier claim,[2] is said to show no sedative activity.[3] Mediodespidine (I), a tetrahydroisoquinoline analogue of reserpine, exhibits a reserpine-like blood pressure drop in Maccacus rhesus monkey given an intravenous dose of 0.5 mg. K. However, no sedative effect was observed until a dose level of 10 mg. K. had been reached.[4] This result is to be contrasted with the statement that I (OCH_2O = 2 OCH_3) is inactive.[5] An excellent summary of the voluminous work on resperine congeners has appeared.[6]



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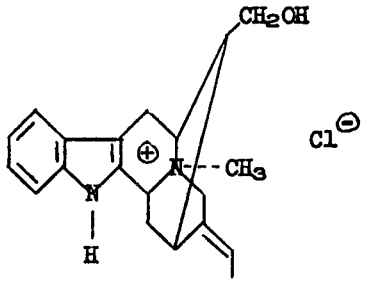


(II)

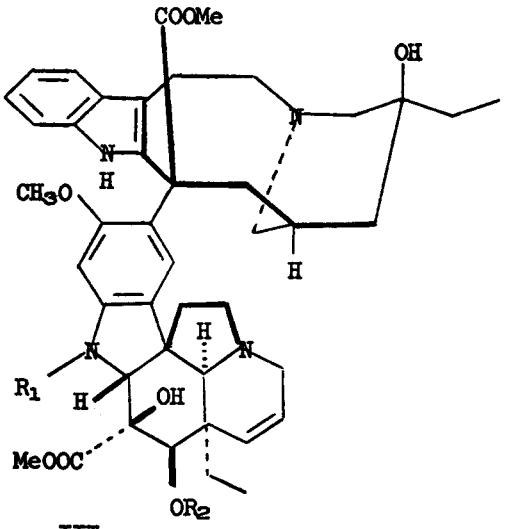
The curare alkaloid, Macusine-B (II) has been shown to block α-adrenergic receptors and to stimulate β-receptors,[7] but is not of practical use.

The activity of Epena snuff powder of the Waica tribe (South American Indians) is due to simple indole derivatives.[8]

The complete structure, stereochemistry and absolute configuration of the antileukemic vinca alkaloids, leurocristine (III); R_1 = CHO; R_2 = $COCH_3$) and vincaleukoblastine (III; R_1 = CH_3; R_2 = $COCH_3$) has been unequivocally established by x-ray analysis of leurocristine methiodide.[9] Clinical success has been achieved with a modified vincaleukoblastine, viz., III (R_1 = CH_3; R_2 = $CO{\cdot}CH_2{\cdot}N(CH_3)_2$ one of a series of compounds prepared from desacetyl vincaleukoblastine (III; R_1 = CH_3; R_2 = H).[10] There was a striking curative effect of this partially synthetic compound on P-1534, therapy being equally effective by the oral or parenteral route. Remissions have been produced in patients with Hodgkins disease and there was a lack of cross resistance between this drug and other vinca alkaloids and alkylating agents.

Mimosine (IV) about 1% in the diet of rats, caused a complete inhibition of the estrous cycle and complete infertility.[11] A normal cycle returned upon cessation of the treatment. It was suggested that an antagonism to vitamin B_6 could account for the above effects.

Injection of lasiocarpine (V) hydrochloride into rats results in a high mast cell count in the para-aortic lymph nodes, a property not shared by the other pyrrolizidine alkaloids tested.[12] The N-oxide reduces mitosis of the parenchymal cells in livers of rats.[13]



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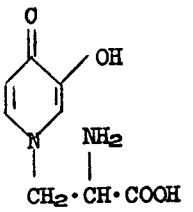

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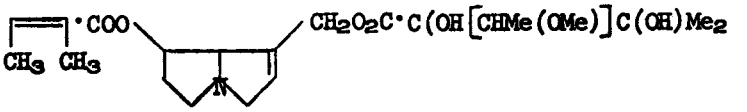

lasiocarpine

V

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Chap. 29 Reactions of Interest in Medicinal Chemistry

by

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Department of Medicinal Chemistry, University of Kansas

The medicinal chemist is interested in the whole spectrum of organic reactions and is usually well versed in the literature of synthetic organic chemistry. This presentation will attempt to treat some new and/or novel reactions which were reported during 1965.

A very interesting book by House[1] appeared during the year and offers a condensed and critical evaluation of modern basic synthetic methods. Since this report cannot be all inclusive the reactions described will be grouped in a classical manner as far as possible.

Methods of reduction are constantly being explored and hydrogenation with homogeneous catalysts have the advantage of being unaffected by the usual catalyst poisons. The reduction can be performed under relatively mild conditions as illustrated by the hydrogenation of methyl linoleate in the presence of iron pentacarbonyl catalyst, $Fe(CO)_5$ to produce the monoene in 65.5% yield at 100° and 400 psi.[2] The homogeneous catalytic hydrogenation of acetylenes gave quantitative yields of the corresponding alkanes.[3] Hydrogenation occurred at less than 1 atm. at 20° using 0.005 mole $(Ph_3P)_3RhCl$, 2 moles of the acetylene all dissolved in a mixture of ethanol-benzene.

Diimide (H_2N_2) reductions have been reported many times prior to this year and continuous work on their selectivity is underway. Diimide was used in the reduction of aldehydes and ketones to the corresponding alcohols. Aliphatic carbonyls were reduced slowly and the yield was low (max 14%), whereas aromatic aldehydes and ketones gave yields as high as 56%.[4]

A new synthesis of N-monosubstituted hydroxylamines involves the reduction of aliphatic ketoximes and aldoximes with diborane. An intermediate is formed which on hydrolysis under acidic or basic conditions gives the hydroxylamines in yields of 50-90%.[5] Nickel borane can be used in selective desulfurization. The yields obtained are comparable to those obtained with Raney-N; in certain instances but in others, such as with p-tolylsulfonates, no desulfurization occurs.[6]

Reduction of ketones with $LiAlH_4$-$AlCl_3$ will give stereoselectivity dependent on conditions. In the mixed hydride reduction under kinetic control, nor-camphor gave 95% of the less stable endo-norborneol and camphor gave 66-71% of the less stable isoborneol, hydride approaching from the less hindered side in both bases. Equilibration of norborneols with excess ketone leads to 89-90% of the more stable exo-isomers. The alkoxyaluminum dichlorides formed from the ketone (or alcohol) and mixed anhydride, in turn function as reducing agents. Isobornyloxyaluminum dichloride is a fairly stereoselective reducing agent in that it produces largely axial alcohols.[7]

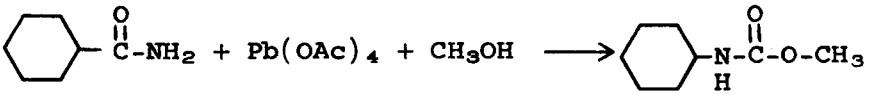
Lithium trimethoxy aluminum hydride is a selective reducing agent which will reduce nitriles and amides to amines but reacts slowly with epoxides.[8]

A good method for the reduction of pyridine N-oxide and its alkylderivatives involves the use of thionyl chloride at 50°. The mixture is allowed to stand 30 min. and on isolation gives the desired amine in 60-85% yield.[9] A new method for the reduction of aromatic nitro compounds with sulfur and sodium hydroxide in an acetone-methanol solution gives high yields of the corresponding amine.[10] Tertiary amides were reduced to tertiary amines with sodium borohydride in pyridine in good yields.[11]

Oxidation of primary and secondary amines with a suspension of argentic picolinate in a polar organic solvent or water yields aldehydes or ketones from most amines. Yields to give aldehydes and ketones were better with secondary amines being in the 30-90% range.[12] This reagent was found to be superior to mercuric acetate or silver oxide. Ozonization of tertiary amines and amides was found to induce a Polonovski reaction. When N,N-dimethylanthranilic acid was ozonized in ethylacetate, methylene chloride, or methanol solution at 0° the demethylated product and the corresponding hydrobenzoxazone were obtained.[13]

Lead tetraacetate was found to react with primary amides to form acylamines.[14] It was suggested that the mechanism involves the formation and rearrangement of an acyl nitrene. The

reaction can be used to form alkyl carbamates if it is performed in the presence of an alcohol.[15] Cyclohexanecarboxamide gave a 96% yield of the methyl N-cyclohexylcarbamate.



The scope of the oxidation of many different types of hydroxyl function with dimethyl sulfoxide-carbodiimide has been studied.[16] The particular utility of the method for the oxidation of primary alcohols to aldehydes and of sensitive molecules such as homoallylic alcohols is emphasized. The method has been successfully applied to alkaloids and to an unusual dehydration of a hemiacetal to give a vinyl ether. Oxidation of benzylalcohol, p-substituted benzyl alcohols, and trans-cinnamylalcohol to the corresponding aldehydes was performed successfully with t-butyl chromate.[17]

Ketones can be regenerated from their p-toluenesulfonylhydrazones by reactions with lead tetraacetate.[18] The reaction in acetic acid at 20° evolves nitrogen and was successful with acetophenone, acetone, cyclohexanone, (+)camphor, (-)piperitone, and benzophenone. Lead tetraacetate was also found to

convert aldehydes in the presence of ammonia to the corresponding nitrile.[19] Best yields were obtained with aromatic

$$RCHO + NH_3 \xrightarrow{Pb(OAc)_4} RC \equiv N$$

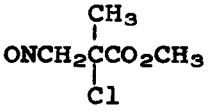
aldehydes. Another convenient one step conversion of aldehydes to nitriles involves the refluxing of a solution of the aldehyde in formic acid with hydroxylamine hydrochloride and sodium methoxide.[20] A number of aldehydes were converted to nitriles by this method and it was found to be particularly successful in the aromatic series.

A new method for halodecarboxylation of acids utilizes lead tetracetate and ionic halide salts.[21] It is a convenient method. A photochemical transformation affords an excellent method for the decarboxylation of acids.[22] This method involves

$$RCO_2H + Pb(OAc)_4 + MX \longrightarrow RX + CO_2 + Pb(OAc)_2 + HOAc + MOAc$$

the photochemical decarboxylation of acylhypoiodites. Primary, secondary, and tertiary acids, as well as the hitherto intractable glutaric and adipic acids, can be decarboxylated without difficulty.

The continuous investigation of various addition reaction gives unique synthetic methods for the preparation of new types of compounds. Phosphorous tribromide can be added to olefins under a variety of conditions to give β-bromoalkylphosphorous dibromides.[23] Nitrosylchloride will react with a variety of unsaturated compounds. Methyl methacrylate was found to give I



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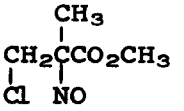
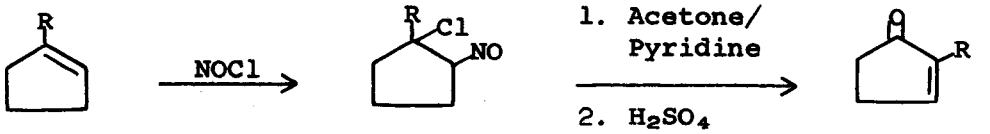


II

when heated with nitrosyl chloride in a sealed tube but II was formed when the reaction was performed in the presence of aluminum chloride.[24] With cyclic olefins the most highly substituted chloride is obtained. The resulting compound can be used for the preparation α-alkylated-α,β-unsaturated ketones.[25]



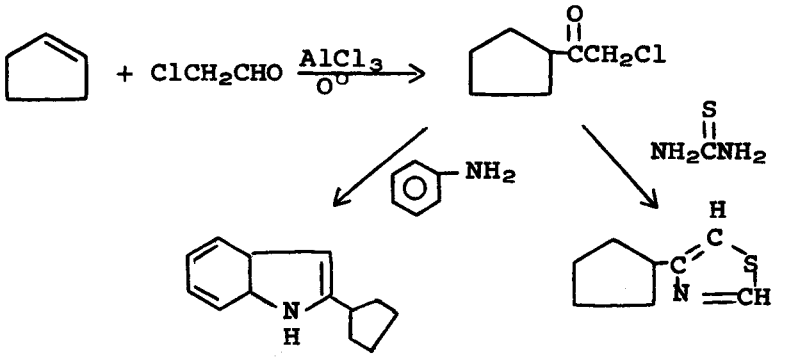
The formation of a new class of aliphatic diazo compounds by the addition of sulfonyl azides ($ArSO_2N_3$) to ethoxyacetylene was reported.[26] The product (III) can be reduced to the corresponding hydrazine, caused to react with acetylenes,

$$RSO_2N{:}C(OC_2H_5)CHN_2$$

III

triphenylphosphine, and mineral acids.[26]

Chloroacetaldehyde will react with olefins in the presence of aluminum chloride. Cyclopentene will undergo this reaction to give an intermediate for the preparation of unique heterocyclic compounds.[27]



The addition of positive halogen compounds can be illustrated by several reactions. Mono and dibromomalononitrile, via a free radical intermediate, will add to aliphatic[28] and aryl substituted olefins[29] in good yields. A novel method for the

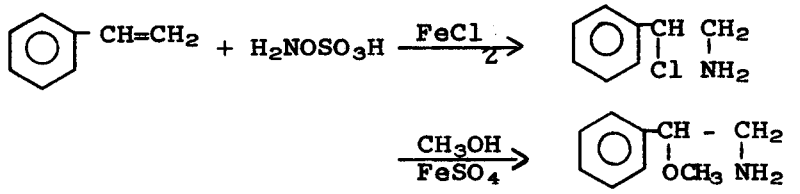
$$CH_3(CH_2)_5CH{=}CH_2 + BrCH(CN)_2 \xrightarrow{Cu} CH_3(CH_2)_5\underset{\displaystyle Br}{\underset{|}{C}}HCH_2CH(CN)_2$$

$$\bigcirc\!\!-CH{=}CH_2 + BrCH(CN)_2 \xrightarrow{Cu} \bigcirc\!\!-\underset{\displaystyle Br}{\underset{|}{C}}HCH_2CH(CN)_2$$

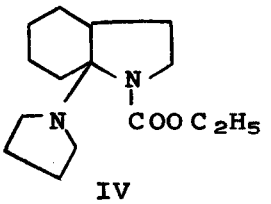
preparation of α,α-dichloro ketones involves the reaction of acetylenes with N-chlorosuccinimide in methanol to give the dihalodimethyl ketals which are readily hydrolyzed with dilute acids to the ketones.[30] The reaction is of general use and affords the ketones in yields of 60-80%.

Enol acetates derived from aldehydes having two α-hydrogens react with bis(1,2-dimethylpropyl)borane. The initial addition involves a slow "anti-Markovnikov" hydroboration but a rapid elimination and rehydroboration occurs.[31] Enol acetates derived ketones do not generally react.

Hydroxylamine-O-sulfonic acid will aminate olefinic, acetylenic, and aromatic compounds.[32]

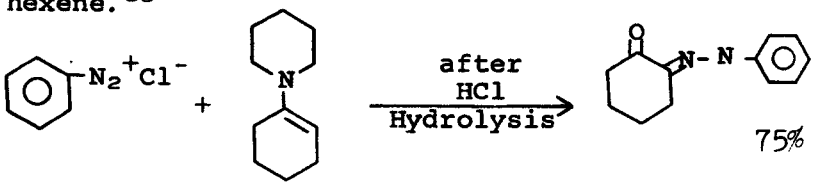


New uses of eneamines have been reported. A new heterocyclic synthesis involving an enamine cycloaddition with N-carbethoxyaziridine and 1-pyrrolidinocyclohexene produced a 42% yield of IV.[33] Dichlorocarbene forms an adduct with the piperdine



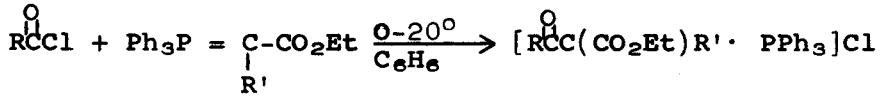
IV

eneamine of cyclopentanone which undergoes ring expansion to 2-chloro-2-cyclohexene-1-one.[34] Ring expansion from the corresponding cyclohexanone adducts was not obtained. Benzene diazoniumchloride was found to react with 1-piperidinocyclohexene.[35]



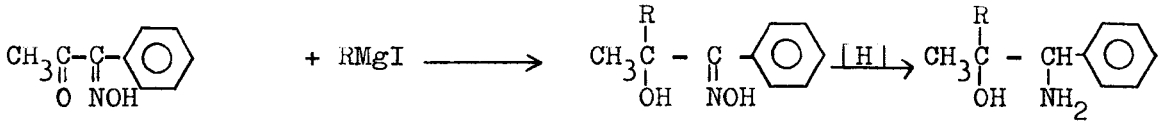
α-Formyl ketones can be prepared from enamines by Vilsmeir formylation. Phosgene, dimethyl formamide, and 1-morpholinocycloalkenes yielded the α-formylketones on hydrolysis.[36]

Phosphorous acid tris(dimethylamide), $[(Me_2N)_3P]$, is suitable in many cases in lieu of triphenylphosphine for the Wittig reaction.[37] The increased rate of formation of the phosphonium salts and the water solubility of the resulting $(Me_2N)_3PO$ formed along with the olefins simplified the preparation of the olefins. The Wittig reaction is used in a new synthesis of α-branched-β-keto esters.[38] The product of the Wittig is

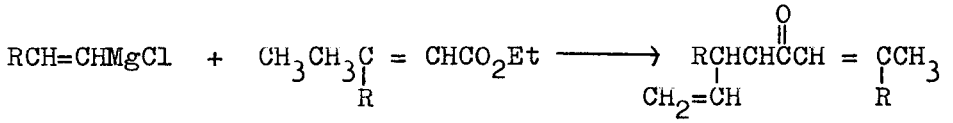


electrolyzed to give good yields of the corresponding ester. Other yields have found wide synthetic use. Dimethyloxosulfonium methylide and dimethylsulfonium methylide are both nucleophiles and both function to transfer methylene to certain electrophilic unsaturated linkages including C=O, C=N, C=S, and in certain cases C=C.[39] A new synthetic route to ketones and "overall methylene insertion" are examples of the use of this system. Carbamyl-stabilized sulfur yields have been prepared via treatment of the corresponding sulfonium salts with sodium hydride. These yields react with Schiff bases to produce 3-aryl amino cinnamate derivatives to demonstrate the synthetic potential of the series.[40]

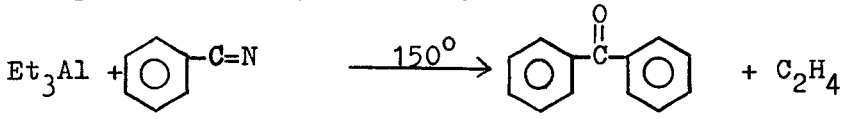
A new synthesis in good yields of amino alcohols with tertiary alcohol groups involves a selective Grignard reaction.[41]



The reaction of vinyl Grignard reagents with α-unsaturated esters gives a preparative method for α,γ'-unsaturated ketones.[42]



Triethylaluminum reacts with benzonitrile.[43] The reaction is believed to proceed through a 6-ring complex utilizing one of the

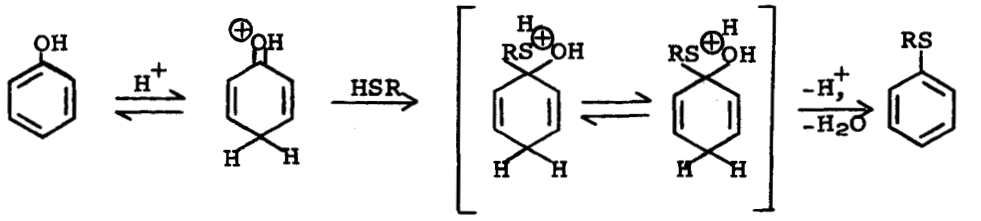


ethyl groups from triethylaluminum for ring formation.

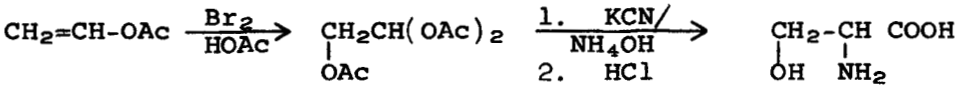
α,α'-Dibrominated dicarboxylic acids can be prepared by bromination in formic acid solution in the presence of red phosphorous and irradiation with a 200 watt lamp.[44] The yields are excellent. A convenient synthesis of cycloalk-2-eneones and α,β,α',β'-cycloalkadienones utilizes the direct bromination of several cycloalkanones in ether followed by dehydrobromination.[45]

Other interesting substitution reactions have been reported during the year. N-formyloxymethyl-N-methylformamide proves to be a useful electrophilic reagent.[46] The acid catalyzed reactions of this compound with carboxylic acids, alcohols, a mercaptan, β-naphthol, anisol, phenol, and thiourea have been studied. All the reaction involve the carbonium ion, $\oplus CH_2NMeCHO = CH_2{=}\overset{+}{N}MeCHO$, as an intermediate. The phenolic hydroxyl group will undergo a nucleophilic replacement by the mercapto group in acid solution.[47] The following mechanism was proposed.

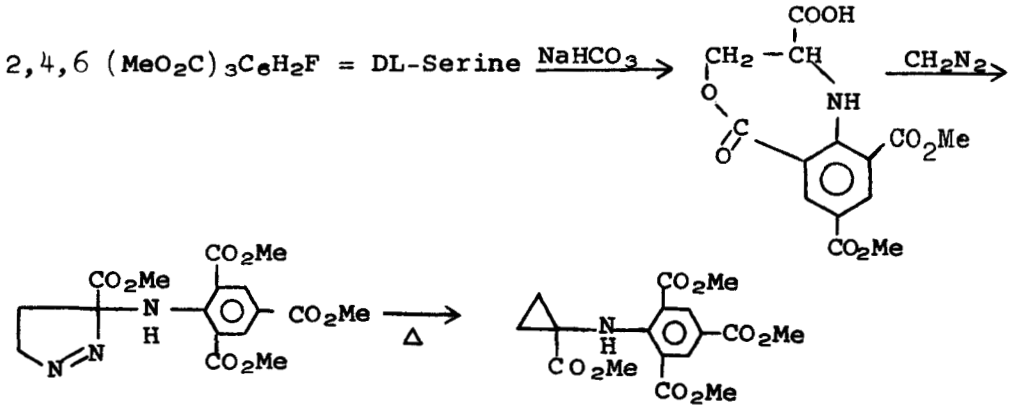
7



A simple synthesis of β-hydroxy-α-amino acids involves a modification of the Strecker reaction.[48]

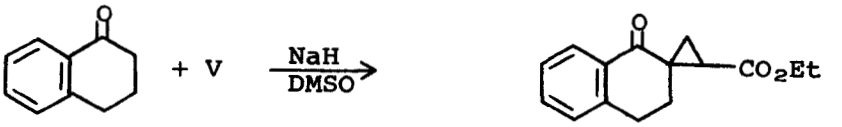


Serine can be converted into a pyrazoline by a novel series of reactions.[49] If the pyrazoline is heated, cyclopropane

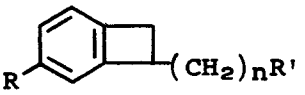


derivatives are obtained. Threonine afforded similar results.

A new method was developed for the synthesis of cyclopropane derivatives by treating phosphonenolpyruvic acid triethyl ester $[(EtO)_2P(O)CH(:CH_2)CO_2Et]$(V) with compounds containing active methylene groups.[50]



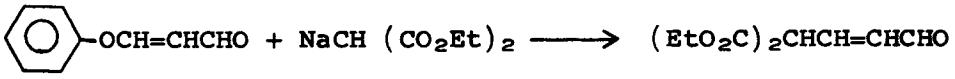
A new series of 1-aminomethylbenzocyclobutenes, VI, was reported,[51] R=H, R'=NH_2, n=0 gave an active analgetic.



VI

Tryptophan can be selectively alkylated at the indole-N position with sodium in liquid ammonia and various alkyl halides.[52] Ethylacetoacetate can be benzoylated by using aluminum chloride.[53] Ethyl benzoacetate is obtained in 45% yield. A method is described for the cleavage of benzyl ethers in molecules containing multiple bonds utilizing sodium and butanol.[54]

β-Phenoxyacrolein will condense with sodio derivatives of active methylene compounds.[55] The latter compound will undergo



various reactions such as alkylation and eneamine formation.

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Chap. 30 ANTIRADIATION AGENTS

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The medicinal chemistry of antiradiation agents is concerned here primarily with the development of new radioprotective agents and their activity. Such a discussion necessarily omits much of the work in radiation biochemistry and contiguous areas, although interesting new developments where chemical interactions have been shown to take place are included. The subject of chemical radioprotection and radiosensitization of tumor-bearing systems has been touched only briefly, covering events subsequent to the reviews cited, and is really worthy of a separate review. Methods of biological radioprotection and clinical aspects have not been covered in this report. Preparations of potentially active antiradiation agents without report of protective activities regretfully have been left for future reviewers when radioprotective results may have become known.

Reviews.- New books concerning chemical radioprotection[1] and the principles of radiation protection[2] have appeared. Current concepts of chemical protection against ionizing radiation have been reviewed,[3,4] and the role of copper and peroxides in radiobiology has been discussed.[5] The 92 papers presented at the First International Symposium on Radiosensitizers and Radioprotective Drugs in 1964 have appeared in a well-organized volume.[6] The use and pharmacology of radioprotective aminothiols, disulfides, nitriles, and other compounds has been reviewed.[7]

Radioprotective Compounds in Mammals.- Chemical modification of the MEA and MEG structures has continued actively, although no real improvement over these two basic structures has been reported in the past two years. A new direction for syntheses of potential radiation protectors appears to be among heterocyclic systems, where protective activity is being found apart from any sulfur content of the molecules. Several non-basic thiols have also been reported active, and sulfur-containing analogs of the amino acids have shown some radioprotective effects. Protection by metal chelating agents, metal chelates, and metal ions themselves has pointed to the importance of the role of metal ions in cellular radiation effects.

Heterocycles. Among a group of 22 thiazolines tested in mice *vs.* 850r (γ-radiation), "aminoethylthiazoline" provided up to 30% protection, presumably because of its ability to hydrolyze to protective fragments *in vivo*.[8] Of 15 imidazoles and related N-heterocycles, benzimidazole gave rats 90% protection *vs.* a lethal dose of x-rays.[9] In a subsequent series of imidazoles and benzimidazoles, 1-naphthylmethyl-2-imidazole provided 100% protection to mice.[10]

3,5-Dimethyl-1-(dimethylcarbamoyl)-pyrazole was found to give 50% protection to mice *vs.* x-rays (LD_{100}).[11] Several C-alkylated thiazolines protected mice against a lethal dose of x-rays, and the activity was comparable to that from the mercaptoamines obtained on hydrolysis of the thiazolines.[12] A copolymer consisting of S-vinyl(2,2-dimethylthiazolidyl)-N-monothiolcarbamate and N-vinylpyrrolidone was also found effective *vs.* x-rays in mice.[13]

Two 5,7-dihydroxyisoflavones, representing a class of compounds for which radioprotective ability has been controversial, were shown to afford 100% protection to mice *vs.* 700r when administered percutaneously but were not protective by the intraperitoneal route.[14]

Thiols. α-Acetamidinium thiosulfates, $RNHC(=NH_2^+)CH_2SSO_3^-$, were radioprotective[15] to mice *vs.* a lethal dose of x-rays, and a substituted 2-aminothiosulfuric acid, prepared from α-aminobutyric acid, also gave good protection.[16] The thiosulfonate of MEA, $NH_2CH_2CH_2SO_2SCH_2CH_2NH_2$, afforded good protection to mice,[17] and it also protected *S. marcescens* at pH 7, where it is decomposed to cystamine and hypotaurine, but not at pH 4, where it is stable.[18] The aminothio acids derived from α- and β-alanine and glycine showed no protective activity in rodents but did in bacteria.[19] Good protection in mice was provided by the β-aminoethylamide of thioglycollic acid, $HSCH_2CONHCH_2CH_2NH_2$, as well as by N,N′-bis(mercaptoacetyl)hydrazine, however, *vs.* 800r (x-rays).[19] N-Acetylthioglycollic hydrazide, $HSCH_2CONHNHCOCH_3$, and its disulfide increased the LD_{50} of x-radiation in mice.[20]

The mixed disulfide of MEA and o-mercaptobenzoic acid was protective in mice *vs.* a lethal dose of x-rays; this compound showed remarkable structural specificity, neither the meta or para isomers nor a variety of other close relatives were protective.[21]

Thiols lacking a basic function also appeared with radioprotective activity. 2,3-Dithiosuccinic acid (both meso and dl forms) protected 90% of mice *vs.* 700r (x-rays).[22] 1-Phenyl-1-acetthio-2-nitroethane, and its next higher homolog, showed some activity in mice *vs.* an LD_{100} dose of x-rays, whereas the corresponding aminothiol, 2-mercapto-2-phenethylamine, was inactive.[23]

Numerous other interesting aminothiol derivatives were synthesized without report of activities. α,α-Dialkyl-β-aminothiols, however, were found inactive in mice,[24] and addition of long chain N-alkyl groups to AET and the trithiocarbonate of MEG, $RNHC(=NH_2^+)CH_2CH_2SC(=S)S^-$, also resulted in loss of activity.[25]

Other Compounds. Hexacoordinated chlorophyllin-metal chelates (with Co,Mg,Mn,V) have been claimed to be radioprotective in mice;[26] and a series of 1,5-diphenylthiocarbohydrazides capable of metal ion chelation provided 40-65% protection to rats *vs.* 750r.[27] An auxin analog, β-2,4,5-trichlorophenoxyethanol,

which reduces normal oxygen consumption, protected mice vs. 800r (γ-radiation).[28] The proestrogen, chlorotrianisene (tri-p-anisyl-chloroethylene), has shown the surprising property of protecting 80% of mice(vs. 590-690r) when administered 5-30 days prior to x-irradiation.[29]

Pyromellitic and benzenepentacarboxylic acids, but not mellitic acid, were protective in mice vs. 1025r when administered in high doses.[30] It was considered that the activity resided in the osmotic effect of these polyionic substances which could cause hypoxia, rather than in the chelation of calcium ions known to occur. Gallate esters, which protected rats vs. 750r, were believed to inhibit chain oxidation processes.[31]

Radioprotection of Other Systems.- Glycine exerted a protective effect on the catalases in mice vs. 500r, but showed little effect on ATPase, pyrophosphatase, or glutaminase.[32] Selenomethionine and selenocystine showed a greater protective effect for amino acids, yeast alcohol dehydrogenase, and RNase than the analogous sulfur compounds.[33] The radioprotective effect of both glycerol[34] and dimethyl sulfoxide[35] for catalase was attributed to complex formation with the iron of catalase. Protection of catalase by Cu^{++}, Fe^{++}, and Mn^{++} ions, however, was explained by radical scavenging.[36]

Iron and copper ions also increased radioresistance of ceruloplasmin and hemoglobin, and a similar effect was shown by Ni-picolinic acid and Ni-glycine chelates.[37] Other chelates did not protect these proteins. A correlation between radioprotection of proteins by inorganic ions and hydration energy of the ions was discerned.[38] Incorporation of iron in erythrocytes was not inhibited by radiation (75r in mice) in presence of MEA, histamine, or serotonin.[39]

Ehrlich ascites cells in mice vs. 400r (γ-rays) were protected by either α- or β-alanine followed by arginine.[40] AET protected both cancerous and healthy cells in mice from x-rays, but did increase the survival rate of cancerous mice.[41] The gastrointestinal tract of rats was protected by perfusion with MEG vs. 900r, but Dunning leukemia cells were not eradicated.[42] The hematopoietic system of mice was protected by cysteine thiosulfonate vs. 500r (x-rays), but Crocker sarcoma cells also received protection.[43] The subject of chemical radioprotection and tumors has been recently reviewed.[44]

Radiosensitizers.- A review has appeared on studies of radiosensitizers in radiotherapy of tumors.[45] Radiosensitization of Ehrlich carcinoma cells by 4-methyluracil and 5-hydroxymethyl-4-methyluracil has been observed,[46] as well as by propyl gallate and other antioxidants.[47] Mice proved to be radiosensitive to 4-deoxypyridoxine, INH, DL-tryptophan, and DL-kynurenine vs. 590r; taurine afforded some protection.[48] Dogs were found radiosensitive to quinoxalinedi-N-oxide, whereas mice had been protected by this compound.[49] Colcemide and urethane increased radiosensitivity in

mice when administered twelve hours prior, but protected when given 48 hours prior to x-irradiation.[50]

Erythrocytes were sensitized by iodoacetic acid and related alkylating agents including ethyl methanesulfonate, iodine, and N-ethylmaleimide.[51] E. coli cells have shown radiosensitivity to β,β -dichlorodiethyl sulfone, whereas the monosulfoxide of cystamine was only slightly sensitizing, and Mg^{++} ions gave some protection.[52] Folic acid analogs[53] and sparsomycin[54] sensitized E. coli to ionizing radiation, and Cu^{++} ions sensitized S. flexneri under anaerobic but not aerobic conditions.[55]

Modes of Radioprotection.- The various mechanisms proposed for chemical radioprotection have been recently evaluated by Bacq.[1] A convincing argument in favor of anoxia was made for the radioprotective action of histamine, acetylcholine, and the catecholamines, but it was believed that the thiols and disulfides protect by interactions with free radicals and oxygen. Arguments in favor of radical interaction, and transfer of energy to sulfur, in combination with mixed disulfide formation, have been advanced recently in regard to protection by thiols.[56] None of the complexities of this problem of explaining radioprotection have been removed by recent results, but it appears pertinent nevertheless to cite evidence supporting some of the current concepts.

No correlation between hypothermia and radioprotection by MEA, cysteine, cyanide, 5-hydroxytryptamine, or diethyldithiocarbamate has been reported from two laboratories;[57,58] both groups conclude that the interaction of MEA, at least, with free radicals constitutes its major protective role. A direct relation between radical inhibitory action and radiation protection was observed for antioxidant phenols, pyridines, and gallic acid esters.[59] A correlation between vasoconstrictive effect and radioprotection was reported for the indole amines.[60]

It has been postulated that the introduction of thiols or disulfides into cells upsets the thiol-disulfide equilibria present, resulting in the increased formation of free thiol groups which can react with radicals. Instantaneous repair of damage by H transfer then takes place.[61] Some evidence for this idea has since been reported. Increased amounts of an endogenous compound with reactive thiol groups has been observed after MEA treatment,[62] as well as an increase in the thiol level of spleen,[63] and the release of intracellular glutathione.[64] The latter compound could eliminate H_2O_2 via the glutathione peroxidase pathway.[64]

The complexation of enzymes by protective agents has been proposed, and evidence for the existence of a protective glycerol-iron-catalase complex has been advanced.[65] Spectrophotometric evidence for the existence of complexation between catalase and MEA, MEG, and diethyldithiocarbamate has been observed, and the subject of metalloenzyme complexation discussed.[66] Radioprotection of lactatedehydrogenase by complex formation with D-lactate has been noted,[67] and decreased catalase inactivation in the presence of oxygen has been attributed to formation of catalase-peroxide complexes.[68]

The problem of energy transfer, from radiation-induced radicals to either vital or protective molecules, lies at the heart of the molecular events taking place in the course of radiation protection. Any of the current hypotheses of radioprotection must ultimately consider this effect. However, some understanding of the process is emerging[56,65,68-71] mainly by use of e.s.r. spectroscopy.

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Chap. 31 PHARMACEUTICS AND BIOPHARMACEUTICS

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The science of dosage forms and their influence on drug absorption and utilization is a rapidly growing medicinal field. In this short review we have attempted to present a limited but representative development in this area occurring within the past two years. The materials treated, admittedly, are in the areas of particular interest to the reviewers.

THERMODYNAMIC ACTIVITY, SOLUBILITY AND COMPLEXING

Although thermodynamic properties of medicinal agents in solution has received relatively little attention in the past, there are a number of reasons why they should be of a major concern to medicinal chemists. One can readily show, for example, a direct relationship between the physiological activities of drugs and their thermodynamic activities at the site of action. One should also note that the rates of drug transport and absorption across membrane relate more to thermodynamic activity than to concentration. Drug solubilities which are inversely proportional to thermodynamic activity coefficient control, of course, rates of dissolution and availabilities of many drugs. And for practical purposes it is more convenient and often necessary in many instances to administer parenteral drugs in solution thus requiring rather sophisticated understanding of solution chemistry to permit dissolution of less soluble pharmaceuticals. For these reasons the nature of the structures of solutions of drugs in aqueous and nonaqueous solvents and the mechanisms of activity of cosolvents in these systems particularly have been recently subjects of increased scrutiny.

Correlation of solubility properties of nonaqueous systems to a single parameter such as dielectric constant has been attempted by several groups in the last two years with limited success[1,2]. A try by Restaino and Martin to fit solubility of benzoic acid in various n-alkanols[3] to Hildebrande's theory of solution proved to be unfruitful. In these instances the solvent phase has been essentially assumed to be a continuum. A more effective recent approach[4,5] has been to look at solute-solvent-cosolvent interactions on a molecular basis, the total solubility of crystalline drug A in solvent B and cosolvent C being represented by a summation

$$\text{Total Solubility} = \sum_{\substack{n=1\\m=0\\p=0}}^{\substack{n=\infty\\m=\infty\\p=\infty}} k_{nmp} A^n B^m C^p$$

At saturation the thermodynamic activity of each species present is equal to that of the solid solute. The total solubility behavior furthermore must take into account the interactions between B and C species.

The mechanism responsible for increases in solubility induced in aqueous solutions by addition of cosolvent continues to be largely associated with formation of solute containing species in these systems. The species may take the form of simple one to one or one to two molecular complexes between the solubilizing agents and the solubilized solutes as reported recently for a series of hydroxyaromatic acid systems[6,7] or less stoichiometrically defined giant aggregates such as micelles or adducts between dispersed polyether surfactants and low molecular weight phenols.[8,9,10,11,12]

A rather interesting instance of solubilization and complex formation was reported by Lach and coworkers[13,14] which apparently involves trapping of organic solutes into cage-like structures of cyclodextrins or Schardinger dextrins in aqueous solutions. The observed stability constants are apparently quite high and the chemical behaviors of the bound species are significantly altered in several instances. There may be some relationship between these and starch adducts reported by Goudah and Guth.[15]

DRUG STABILITY

Increasing attention has been directed towards elucidation of mechanism responsible for deterioration of medicinal preparations. Since drug formulations often involve relatively complex mixtures of polyfunctional organic and inorganic species, chemistries of their breakdown on storage under varying conditions for periods of months and years can indeed be quite involved. Mechanisms of degradation of cycloheximide[16] of porfiromycin;[17] of hydroxocobalmin,[18] of Mannich compounds,[19] of diaminotriazine derivatives,[20] of apomorphine,[21] of iodoxuridine,[22] and of methicillin[23] are representative of some of these systems recently studied. Attention should be called to series of papers on an anaerobic loss of ascorbic acid.[24]

Steroids continue to receive attention. Although prednisolone in aqueous solution undergoes oxidative breakdown catalyzed by metals,[25,26] Jensen and Lamb have shown that autoxidation of fluprednisolone acetate is preceded by a hydrolytic step.[27]

Participation of other ingredients in formulations affecting drug stability has become increasingly evident. Citrate and tartrate buffers, for example, have been shown to be in slow equilibria with their corresponding acid anhydrides which can react rapidly with any nucleophile which may be present.[28,29,30] Waake and Guttman,[31] on the other hand, have found that formation of a borate complex tend to stabilize riboflavine. Lach in his studies on cyclodextrins have shown that these form complexes with drugs often conferring great atability to the bound guest.[32]

PHARMACEUTICS OF HETEROGENEOUS SYSTEMS

Powders, Suspensions and Emulsions: Rippie and his collaborators have reported[33,34,35] some noteworthy studies on the segregation kinetics of particulate solids. These investigators have taken steel and glass balls of various sizes loaded into cylindrical containers and subjected them to vertical sine wave motion. Samples were taken at various times and standard deviations from the mean composition were determined. Segregation of binary mixtures of spheres followed an apparent first order approach to equilibrium.

Employing this technique the authors have studied the influence of particle size, size distribution, particle density-size interactions, and the dependence of the agitation upon the segregation rate. Other variables such as particle shape could be studied also. While the idealized experiments have not included (and may not be able to include) some of the factors important in real systems, e.g., particle-particle adhesion, particle wall adhesion, and electrostatic effects, they have quantitatively clarified many aspects of the particulate mixing phenomena and demonstrated the importance of this kind of approach.

Shlanta and Milosovich[36] have described an apparatus for studying stress relaxation of powder beds under constant strain. The technique appears to be useful for studying elastic compressibility, relaxation and flow under pressure - factors that are basic to the understanding of the tabletting process.

The Coulter Counter played an important role in research on emulsions and suspensions of pharmaceutical significance. Lemberger and Mourad[37,38] studied the influence of a number of variables on the deaggregation behavior of oil-and-water emulsions. Rowe,[39] also employing the Coulter Counter, studied the effect of emulsifier type and concentration on the particle size distribution of oil-in-water emulsions. Edmundson and Lees[40] determined the dissolution rate of a fine suspension of hydrocortisone acetate in water using the Coulter Counter. These and other work[41,42,43] with

this instrument involving emulsions and suspensions point out the value of this tool in future work with pharmaceutical dispersed systems.

Dissolution Rate Behavior: A number of studies on the rate of dissolution have been recently reported. Wurster and his associates[44,45,46] have studied the effect of complex formation on the dissolution rate of drugs and the dissolution behavior of the three different crystalline forms of prednisolone. In the latter study these investigators observed the unusual effect of agitation on the relative dissolution rates of polymorphs first reported by Hamlin et al.[47]

The dissolution rate behavior of polyphase systems has recently received the attention of several workers since the report by Sekiguchi and Obi[48] that the eutectic mixture of sulfathiazole and urea gives a much higher drug release rate than the pure drug alone. The authors had attributed the greater rate to the smaller crystals in the eutectic mixture. In their recent work with the chloramphenicol-urea system, Sekiguchi et al.[49] found that the drug-urea mixture of 1:4 (W/W) dissolved significantly faster than the eutectic composition. The authors proposed that urea solubilization of the drug was probably the more important factor. Goldberg et. al.[50] has suggested the possibility of solid solution formation in these systems as an alternative explanation. Higuchi et. al.[51] have presented a mathematical analysis of dissolution rates involving polyphase mixtures which should be helpful in resolving the various factors.

BIOPHARMACEUTICS

Current thinking in regard to the absorption of drugs is based largely on the early studies of Overton[52] and Collander and Barlund[53] who have advanced the concept of the lipoidal nature of the biological membrane and more recently upon the work of Schanker, Brodie, Hogben and collaborators[54,55,56,57,58] who have defined the role of lipid/water partition coefficients, ionization and pH in the passive transport of drugs and other foreign compounds across biological membranes. These studies have shown that most drugs are absorbed by passive transport and that the rate of absorption is a function of the concentration gradient of the diffusing moiety across the membrane which in turn is dependent upon the lipid/water partition ratio of the diffusing moiety . The diffusing moiety for weak electrolytes is the non-ionized portion of the dissolved drug. Passive absorption of lipid-insoluble molecules occurs through small aqueous channels or pores in the membrane if the molecules possess sufficiently small molecular volumes to permit passage through the pores. The transport of highly ionized, lipid-insoluble compounds, such as the quaternary amines, is not satisfactorily explained by the above mentioned lipid-partition theory. Levine[59] has postulated that the transport of quaternary amines

may be dependent upon the interaction of these highly ionized molecules with a phosphatido-peptide constituent of the membrane and it is this complex that is the diffusing moiety in the transport of quaternary amines across biological membranes. Such an interaction satisfactorily explains the rapid, but short lived oral absorption characteristics of the quaternary amines observed in man and other animals. The mechanism of permeation of biological membranes has been the subject of several recent reviews.[60,61,62,63]

From the biopharmaceutical standpoint, the absorption of a drug into the body encompasses other parameters in addition to the permeation of the biological membrane. Included among these are dissolution properties (rate of dissolution and absolute solubility), drug interactions affecting drug availability and diffusion of the drug from its site of dissolution to the absorptive surface. These parameters will constitute the subject material to be discussed in the following paragraphs.

Rate of Dissolution and Drug Absorption: Both absolute solubility and the rate of solubility are important parameters in the overall absorption processes. It has long been known that a compound must possess sufficient aqueous solubility to be effectively absorbed from the gastro-intestinal tract as well as other obsorptive sites. More recently, Nelson[64] has clearly elucidated the role of the rate of solubility in the absorption process and pointed out the theoretical relationships that exist between the rate of dissolution of a particle and its surface area, diffusion layer pH and the concentration gradient of drug across the diffusion layer. The rate of dissolution principles have provided the theoretical bases for many recent biopharmaceutical studies.

The effect of particle size of sulfisoxazole on its oral absorption in dogs has been studied by Fincher, Adams and Beal.[65] These authors concluded that the rate of absorption of sulfisoxazole was a function of the particle size of the crystals administered, faster and higher blood levels of the drug being obtained with the smaller crystal sizes. Their results indicate that while the rate of absorption was affected by particle size, there was no alteration in the total percentage of administered dose absorbed thus giving rise to the possibility that the blood levels of sulfisoxazole could be controlled by regulating the particle size of the administered drug.

The rate determining step in the absorption of salicylate from pharmaceutical dosage forms appears to be the dissolution process.[66,67,68] More recently, Truitt and Morgan[69] have studied the absorption of buffered and non-buffered dosage forms of acetylsalicylic acid in humans and have related their in vivo findings to differences in dissolution rates of the administered aspirin. Their results indicate that (a) statistically significant differences in the rate of aspirin absorption from buffered and non-buffered tablets do exist and (b) the enhancing effects of buffering are primarily upon the rate of dissolution of the acetylsalicyclic acid.

Thus, when the buffered aspirin formulation was administered as a slurry or as a solution, buffering had no enhancing effect upon the overall rate of absorption of acetylsalicylic acid. Only when the tableted form of aspirin was administered did the enhancing effect of buffering appear, suggesting that the role of the buffer is to alter diffusion layer pH and thus increase the dissolution rate of the aspirin. The importance of dissolution rate on the rate of absorption of salicylate was also shown by Lieberman and Wood[70] who found that higher blood salicylate levels and more rapid absorption of salicylate occurred when the analgesic was administered in the form of a solution than when administered as either a buffered or non-buffered tablet. Their data indicate that buffering enhanced the overall absorption rate of acetylsalicylic acid when administered in tablet form.

Levy and Hollister[71,72] were also able to show a relationship between the rate of dissolution and the rate of absorption of acetylsalicylic acid in humans. These authors employed both conventional compressed tablets and an experimental sustained release preparation employed produced a significantly reduced dissolution rate as compared to the dissolution rate obtained for the compressed tablets. Similarly, the first order rate constants for absorption following the administration of the sustained release from were significantly lower than those obtained with the compressed tablets. In addition, the sustained release dosage form caused a delay in the onset of absorption in most patients. Despite the delay in onset of absorption and the much slower rate of absorption, the total amount of salicylate absorbed from the sustained release dosage from compared favorably with that obtained from the conventional tablet. These results indicate again the role of the rate of dissolution in the over-all absorption rate of acetylsalicylic acid.

The importance of rate of dispersion and dissolution of a drug was made evident by the work of Calesnick, Katchen and Black[73] who administered various dosage forms of diazoxide and obtained blood levels and biological response data in humans. Comparing tablet and capsule forms with an aqueous solution of the drug, these authors found the highest blood levels were obtained when the aqueous solution of diazoxide was administered, lowest blood levels being obtained with the tableted form and the administration of the encapsulated drug producing blood levels intermediate between those obtained with the solution and tablet forms. It was evident from their data that the dispersion of the formulation contained within the capsule was the rate determining step in the dissolution of the drug when utilized in this form. Very rapid dissolution rates were observed when the capsule contents were emptied and evenly dispersed throughout the dissolving media as compared to the very slow dissolution rate observed when the intact capsule was placed in the media. Similar findings in regard to blood

levels obtained with encapsulated aspirin vs. tableted aspirin were obtained by Wood.[74] In this study, a significant delay in the appearance of salicylate in the blood was observed in those persons receiving aspirin in the form of capsules as compared with those receiving the analgesic in the form of tablets. In addition, the blood levels of total salicylate were lower in the group receiving the capsules than was found in those receiving the aspirin in the form of a tablet. Hollister and Kanter[75] compared enteric coated dosage forms of aspirin with compressed tablet preparations. They observed significant delays in onset of serum salicylate levels when the enteric coated preparations were employed. The delay in absorption found in their study was of such magnitude (three to six hours) that they concluded that enteric forms of salicylate should not be employed where an immediate analgesic or antipyretic effect was desired.

Percutaneous Absorption: The influence of solubility and lipid/water partition ratios in the percutaneous absorption of the corticosteroids were studied by Katz and Shaikh.[76] The results of their study are in agreement with the theoretical postulates of Higuchi and their results show that the efficiency of percutaneous absorption absorption of the corticosteroids may be a function of the partition coefficient and the square root of the aqueous solubility. In their continuing studies pertaining to the factors influencing percutaneous absorption, Wurster and Munies[77,78] have shown that the degree of hydration of the stratum corneum played an important role in the transport of methyl ethyl ketone. These workers have shown that dehydration delays percutaneous absorption while hydration of the skin above normal values enhances absorption. Thus, percutaneous as well as oral absorption of chemical substances appears to be a function of the lipid/water partition coefficient and aqueous solubility parameters of the diffusing moiety and intimately associated with the integrity and physico-chemical properties of the biological membranes.

Drug Interactions: The interaction of dissolved drugs with either endogenous or exogenous materials present in the vicinity of the absorptive site may have a pronounced effect on the absorbability of the drug. Interaction of the drug with these materials may either facilitate or inhibit drug absorption. Sorby[79] has shown that promazine adsorbed to the surface of both attapulgite and activated charcoal and this interaction of drug and adsorbent resulted in altered absorption patterns of promazine. When admixed with attapulgite, the absorption of promazine was significantly delayed, however, only minor decreases in the proportion of the administered dose absorbed was observed. On the other hand, when admixed with charcoal, the drug-adsorbent interaction resulted in both a decrease in the rate and the extent of absorption of promazine. Although absorption characteristics were not studied, Blaug and Cross[80] investigated the adsorption of antichlolinergic drugs by

various antacids and found appreciable binding of atropine, methantheline, propantheline and oxyphenonium to the antacids, particularly magnesium trisilicate. In lieu of the frequent coadministration of anticholinergic drugs and antacids, definitive in vivo absorption studies are necessary to define the possible effects of the antacids on the therapeutic efficacy of the anticholinergic drugs.

The intestinal absorption of water soluble dyes such as bromthymol blue, methyl orange and eosine-B and certain lipid soluble complexes of the dyes have been investigated by Levy and Matsuzawa.[81] Their data suggests that the lipid/water partition ratio of the dye complexes did not reflect the intestinal absorption characteristics observed. The absorption characteristics of certain metal-acid complexes of tetracycline and demethylchlortetracycline have also been investigated and it has been shown that some of the metal-acid-tetracycline complexes enhance the absorption of the tetracyclines.[82]

Diffusion: Diffusion of the drug from the site of dissolution to the absorbing surface may have a pronounced effect on the maintenance of the concentration gradient across the absorbing membrane and thus an effect on the rate of absorption. This parameter of the over-all absorption process was the subject of a report by Levy and Jusko.[83] These workers studied the oral absorption characteristics of both ethanol and salicylic acid in rats as a function of the viscosity of dissolving media. Their results indicated that diffusion of the drug molecules to the absorbing membrane was significantly decreased and the rate of gastrointestinal transit of the solutions was also decreased.

Urinary Excretion: In lieu of the frequent use of urinary excretion data as an index of drug absorption and elimination rates in the evaluation of pharmaceutical dosage forms, it would appear to be of value to call the reader's attention to the recent works of Beckett et al.[84,85,86] pertaining to the excretion of drugs in man. These workers have investigated the influence of urinary pH and urine output on the renal excretion of several drugs. In regard to their studies on the excretion of amphetamine,[84] the authors state that the alterations in excretion rates observed as a function of urinary pH and urine volume can be explained on the basis that the unionized portion of the amphetamine is reabsorbed by the Kidney and the passive reabsorption process is pH and volume dependent. The more alkaline the urine, the higher the percentage of unionized amphetamine present and hence a greater reabsorption of the drug and consequently a lower excretion rate. Similar findings were reported by Beckett and collaborators for chlorpheniramine[85] and methylamphetamine.[86] While these studies are largely applications and extensions of the basic studies reported much earlier,[87,88,89,90] the kinetic approach to data evaluation and implications deduced from their findings throughout their reports are indicative of the value of these reports to the biopharmaceutical literature.

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