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PREFACE

Readers of this current volume may note that several chapters mention that, in the field discussed, there were no real breakthroughs reported during 1970. This may well characterize the whole area of Medicinal Chemistry at present. "Where are the new drugs?" has been used as a title of several talks and articles, but no definitive answer has been forthcoming.

However, I believe encouragement can be found in a number of chapters which discuss significant new contributions to our understanding of normal and abnormal physiological function and of the mechanism of drug action. Interesting theories have been advanced which may pave the way to new breakthroughs.

The problem of adequately thanking editors, authors and other contributors to this volume is no easier than for previous volumes. The many favorable comments, reviews and personal communications received indicate general gratitude. I can only add that former contributors can best appreciate the efforts expended -- and they do.

Cornelius K. Cain

Section I - CNS Agents

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Chapter 1. Antipsychotic and Anti-anxiety Agents

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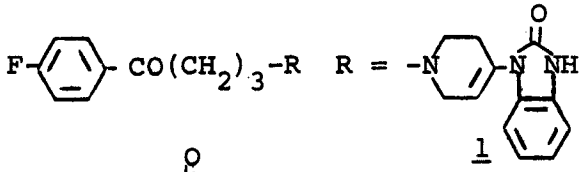
Introduction - As in previous years, molecular modification of known structures, especially in the tricyclic systems, has played an important part in the chemistry and pharmacology of neuroleptic drugs. Most of the reported work on benzodiazepines was concerned with additional studies of compounds already undergoing clinical investigation. There appeared to be less emphasis on the chemistry and pharmacology of the carbamates and little new was added to the knowledge of mechanisms and modes of action of antipsychotic and anti-anxiety agents.

Compounds Related to Butyrophenones -



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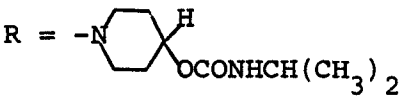

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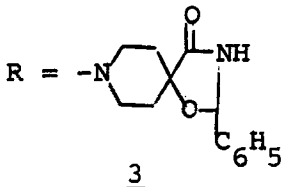

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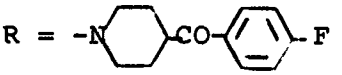

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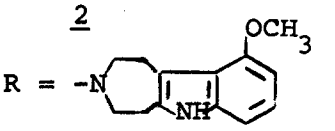

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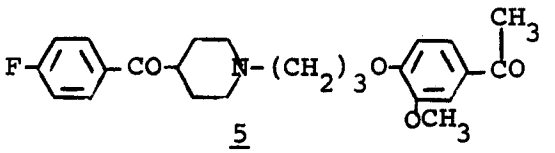

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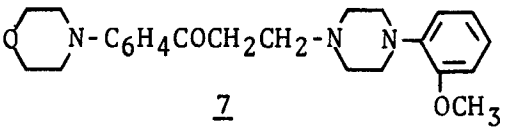

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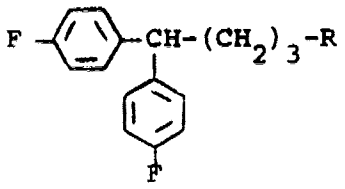
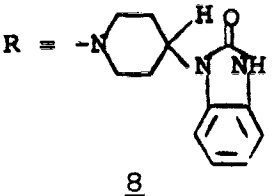
A double blind controlled study showed an approximately 10% better response in schizophrenic patients with droperidol 1 than with haloperidol.[1] Pilot studies in schizophrenic patients with AL 1021 (2) indicated the drug to be a potentially useful antipsychotic agent. The chemistry of this and related compounds have been reported together with pertinent references to the clinical studies.[2] The synthesis and

pharmacology of a series of spirooxazolidinone substituted piperidinobutyrophenones (e.g. 3) were reported. Although spatially related to spiperone, the animal pharmacology indicates a neuroleptic profile nearer to that of chlorpromazine than to that of the butyrophenone derivatives.[3] Two of the most active of a series in a new class of potent CNS depressants are exemplified by structures 4 and 5. The preparation and animal pharmacology of a total of 57 related compounds are reported.[4] Compound 6 was the most active of a series of related butyrophenone derivatives (2x thoridazine in mouse behavorial tests with a better therapeutic index).[5] Initial clinical trials carried out with SU 17595A (7) indicated only a very slight psychotropic activity. It would not be considered a drug of choice in the treatment of severe symptoms of psychosis.[6]





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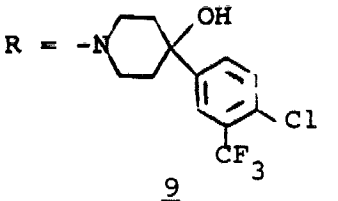

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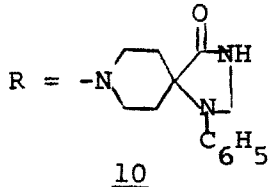

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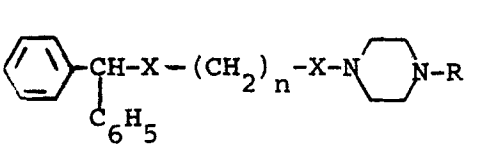
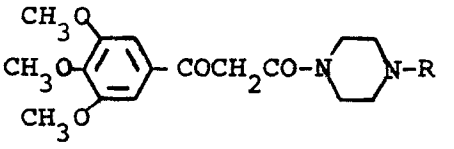
Pimozide (8) was compared to fluphenazine in a study in chronic schizophrenic patients.[7] An additional clinical paper also reports pimozide as a major neuroleptic for the treatment of delusions and hallucinatory behavior.[8] The pharmacology of the related penfluridol (R 16341, compound 9), a new potent and long acting neuroleptic drug has been reported.[9] Maintenance therapy with penfluridol in a double blind study was shown to be effective with a single weekly oral dose.[10]

In an additional study, fluspirilene (10), administered intramuscularly in aqueous suspension, at weekly intervals was an effective antipsychotic agent. Side effects were noted in 70-75% of the patients.[11] The pharmacological profile was that of typical known neuroleptic compounds.[12] A single weekly dose of fluspirilene was better or equal to a daily dose of haloperidol in 79% of schizophrenic patients.[13]



X = N, O, S, CO, CH_2

11



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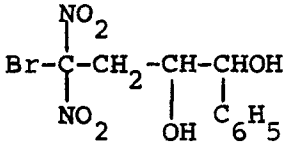

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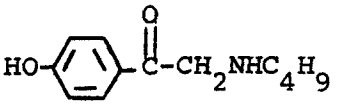
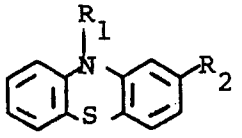


14

A series of 93 compounds of type 11 was prepared and tested. Some compounds exhibited signs of CNS depressant activity in the mouse. Other compounds showed antihistaminic properties.[14] Also synthesized and examined pharmacologically were a large number of compounds related to 12. Few of the compounds exhibited any significant CNS activity.[15] An initial report on the animal pharmacology of 13 indicated the compound to be a neuroleptic with medium toxicity.[16] The pharmacokinetic properties of haloperidol[17] and trifluperidol[18] were studied in the rat. The pharmacology of a new neuroleptic agent, BON (14) was covered in a series of reports.[19]

Tricyclic compounds with six-membered rings -



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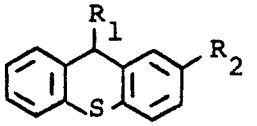


16

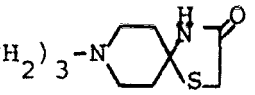
17

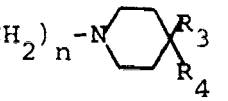
a) R_1 = $(CH_2)_3$-N⟨⟩N-CH_3 R_2 = $SO_2N(CH_3)_2$

b) R_1 = $CH(CH_2)_2$-N⟨⟩N-CH_3 R_2 = $SO_2N(CH_3)_2$

c) R_1 = $(CH_2)_3N(CH_3)_2$ R_2 = Cl

d) R_1 = $(CH_2)_3$-N⟨⟩-OCH_3 R_2 = $COCH_3$

e) $R_1 =$  $R_2 = Cl$

f) $R_1 =$  $R_2 = Cl, CF_3, OCH_3, SCH_3$; $R_3 = OH, CN, CONH_2$; $R_4 = C_6H_5$, NC_5H_{10}

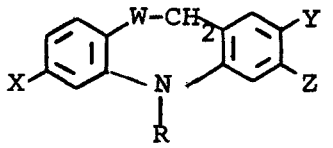
A large number of papers appeared this year on some of the older phenothiazine type drugs (e.g. mesoridazine, flupenthixol, chlorimpiphenin, clopenthixol, propericiazine, thiothixene). Few of these current reports add to our knowledge on the efficacy or mode of action of these drugs.

An attempt to reduce the extrapyramidal side effects of the phenothiazines by the incorporation of anticholinergic properties into phenothiazines was reported. This was effected by the preparation of quaternary salts of chlorpromazine, trifluoperazine and perphenazine by the action of the oxime of phenacyl bromide on these compounds. Pharmacological results with these quaternary derivatives indicated a general loss of overall activity with a greater supression of extrapyramidal effects than loss of CNS depressant properties.[20, 21] The chemistry of thiothixene 16b and its related saturated analogue 16a was reported as the outcome of a search for compounds related to thioproperazine 15a (and chlorpromazine 15c) with less extrapyramidal side effects.[22, 23] Benzoctamine 17 was reported to be an antianxiety agent equivalent to diazepam[24] with little if any antipsychotic activity.[25] Other reports on this drug reported drowsiness and lethargy as side effects.[26, 27] A new piperidylphenothiazine derivative A-124 (15d) was reported clinically to be a mild neuroleptic agent with only some instances of mild side effects.[28] Metabolism, absorbtion, distribution and pharmacological studies in animals were reported for the new neuroleptic agent, APY-606, compound 15e.[29] APY-606 has a potent sympatholytic action with low toxicity. The compound was reported to have a spectrum of pharmacological activity different from that of either chlorpromazine or thoridazine.

The synthesis and pharmacology of a series of 2-hydroxy and 2-alkoxy thioxanthene derivatives has been described.[30] Additional phenothiazines related to 15f substituted with an alkypiperidyl group were also prepared and reported to have CNS depressant activity.[31]

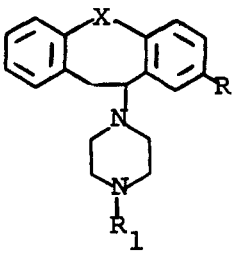
Tricyclic Compounds with a seven-membered ring -

The results of several additional clinical studies of octoclothepine in patients with schizophrenic psychoses were reported this year by several different authors in the same journal.[32] The related clotiapine was reported to be an excellent drug for the treatment of excitation or agitation.[33]



18 W = O or S

A series of 54 dihydrodibenzoxazepines and dihydrodibenzothiazepines related to 18 were prepared.[34] The pharmacological profile of these compounds indicated that when R was a dialkylaminoalkyl group, the compounds were CNS stimulants at high doses. However, when R was the 3-[1-(2-hydroxyethyl)-4-piperazinyl]propyl group, the compounds were CNS depressants at high doses and showed antianxiety effects at lower doses. A number of compounds (19) related to known neuroleptic agents were prepared and evaluated. Trifluthepin was reported to have the pharmacodynamic profile of a potent



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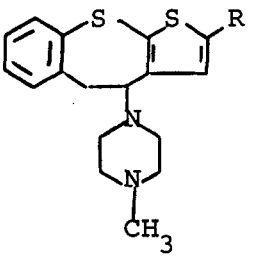
19a) X = S, R = CF_3, R_1 = CH_3 (trifluthepin)
19b) X = S, R = H, R_1 = CH_3 (perathiepin)
19c) X = S, R = Cl, R_1 = CH_3 (octoclothepin)
19d) X = S, R = CN, R_1 = CH_3
19e) X = O, R = Cl, R_1 = CH_3 (loxepine)

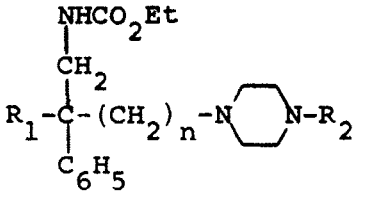


20a, R = Cl
20b, R = H

neuroleptic.[35] The selenipin derivatives corresponding to perathiepin and octoclothepine were prepared (19, X = Se). The central depressant activity of these compounds was less than that of the corresponding thiepins.[36] The therapeutic index of 19d was less favorable than for that of octoclothepine, but better than for that of perathiepin.[37] Oxepin analogues (19, X = O) also synthesized were less active than the corresponding thiepins.[38] One clinical study carried out on loxepine (19e) doubted the efficacy of the drug as an antipsychotic[39], while other workers in different studies reported the compound to be a useful and effective drug.[40] The syn-

thesis and pharmacological evaluation of a substituted derivative of peradithiepin, compound 20a was compared with peradithiepin itself (20b).[41]

Carbamates - Evidence has been reported that the major site of action of tybamate is at the spinal level. A somewhat lesser influence may simultaneously be exerted on the higher centers (the major site of CNS depressant activity of diazepam was shown to be at the brain stem level).[42] In a study of the action of tybamate, it was concluded that this drug does not possess the anxiety-reducing properties to a degree that can be observed with the benzodiazepines.[43] A series of 44 basic dicarbamates related to $X[(CH_2)_nOCONHR]_2$ were prepared and pharmacologically screened.[44] Studies in mice indicated that one compound had CNS depressant properties, while another exhibited antidepressant activity. Twenty-nine compounds related to 21 were prepared. None of these compounds exhibited any worthwhile behavioral or anticonvulsant effects.[45] Pharmacological study of a series of twenty-six derivatives of 1-propargyl-2-carbamoylglycerol ethers (22) indicated that certain of these might have value as tranquilizers and muscle relaxants.[46]



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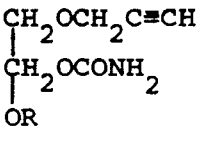
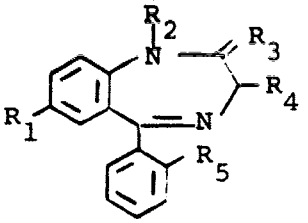


22

Compounds Related to Benzodiazepines -



23, R_1 = Cl; R_2 = $CH_2CH_2N(C_2H_5)_2$; R_3 = O; R_4 = H; R_5 = F

24, R_1 = Cl; R_2 = CH_2  ; R_3 = O; R_4 = H; R_5 = H

25, R_1 = Cl; R_2 = CH_3 ; R_3 = H_2; R_4 = H; R_5 = H

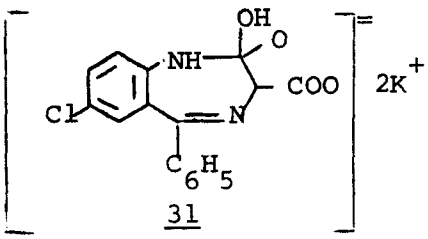
26, R_1 = Cl; R_2 = H ; R_3 = O ; R_4 = OH; R_5 = H

27, R_1 = NO_2; R_2 = H ; R_3 = O ; R_4 = H ; R_5 = H

28, R_1 = Cl ; R_2 = H ; R_3 = O ; R_4 = H ; R_5 = NO_2

29, R_1 = NO_2 ; R_2 = CH_3 ; R_3 = O ; R_4 = H ; R_5 = F

30, R_1 = Cl ; R_2 = CH_3 ; R_3 = O ; R_4 = H ; R_5 = H



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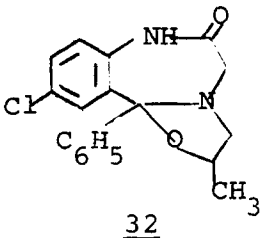

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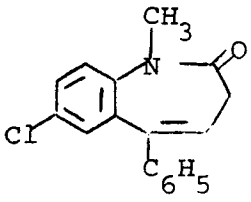


33

Flurazepam (23) was marketed in the United States in 1970 as a hypnotic. Animal pharmacology has been reported.[47] The metabolism of flurazepam in both dogs and humans was investigated.[48] All of the metabolites identified either showed modification of the diethylaminoethyl side chain or lacked the 1-substituent altogether. The major metabolite in man was the 1-(2-hydroxyethyl) compound while in the dog, the corresponding 1-(acetic acid) predominated. Several additional reports on prazepam (24) appeared during 1970. These included metabolism in man[49] and in the dog[50], animal pharmacology[51], and a double blind controlled study in psychoneurotic patients.[52] Medazepam (25) was reported effective in the treatment of anxiety associated with somatic diseases.[53, 54] A double blind crossover study with phenobarbital and medazepam showed significant differences favoring medazepam.[55] The animal pharmacology of the drug and its metabolites was reported[56] as were studies in cats on the neuropharmacological action of the compound.[57, 58] Oxazepam (26) was reported to markedly improve the disturbed day sleep of night workers, but did not effect a normalization equivalent to night sleep.[59] The *in vivo* reduction of the nitro group in nitrazepam (27) is well known. This reduction has now been investigated with tissue preparations *in vitro*.[60]

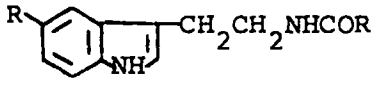
Clonazepam 28 and Ro 5-4200 29 were reported to be more potent (5x) than nitrazepam and diazepam (30) in their anti-epileptic and antiphotoconvulsive properties. Clonazepam, better tolerated than Ro 5-4200, would be the drug of choice.[61] A preliminary clinical study indicates clonazepam to be a valuable drug in the management of minor motor seizures.[62] Several pharmacological studies in animals were also reported

on this compound.

A number of reports on pharmacological[63], toxicological, teratological[64], neurophysiological and clinical studies on chlorazepate (31) appeared this year. A summary of four years experience with the drug by one investigator, showed its effectiveness as a minor tranquilizer.[65] Animal pharmacology of oxazolazepam (32) was reported.[66, 67]

Additional reports on the synthesis and pharmacological activity of 1-substituted 1,4-benzodiazepines have appeared this year.[68, 69, 70, 71, 72, 73] The mass spectral fragmentation pattern of 1,4-benzodiazepine derivatives has been elucidated.[74] In one article, x-ray analysis was used in an attempt to relate molecular structure to the anticonvulsant activity of diazepam and diphenylhydantoin.[75] The authors fail to discuss the steric conformation of the less active deschloro analogue. A study of the structural activity relationships of 1,4-benzodiazepines based on molecular orbital calculations was reported in a lecture.[76] The mode of action of chlordiazepoxide, diazepam and nitrazepam has been suggested to involve catecholamines in the CNS.[77] A benzazepinone (33)[78] was synthesized and found to be inactive. The synthesis (and in some cases, the animal pharmacology) for diazepine analogues in which the fused benzene ring has been replaced by a hetero ring have appeared in the patent literature.[79]

Other structures of current interest -



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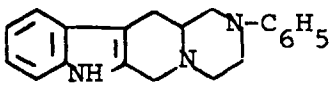

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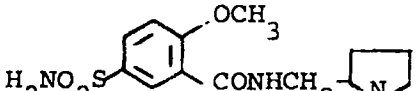

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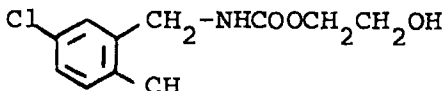

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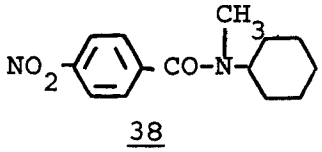
A series of diamides of cyclobutane-1,1-dicarboxylic acid were prepared and reported to have depressant properties.[80] The activity of bis(1,1,1-trichloro-2-propyl)-1,2-cyclobutane-dicarboxylate was shown to be due to the *in vivo* hydrolysis to the known hypnotic 1,1,1-trichloro-2-propanol.[81] Preliminary pharmacological results in mice indi-

cate that some N-pyridoyltryptamines (34) were CNS depressants.[82] The synthesis of 35, structurally related to oxypertine, was reported.[83] No CNS activity was detected in mice. Sulpiride, 36, was reported to prevent the formation of gastric ulcers in rats.[84] Several additional reports demonstrating the clinical efficacy of both molindone[85] and oxypertine[86] appeared this year. An additional favorable study on trioxazine with psychoneurotic patients was carried out.[87] Compound 37 was the most active of a series of α-amino-o-xylenes prepared. This compound was reported to have the pharmacological spectrum of a minor tranquilizer.[88]



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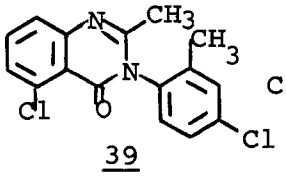

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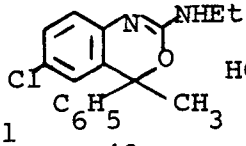

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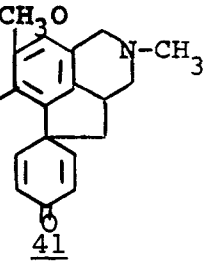

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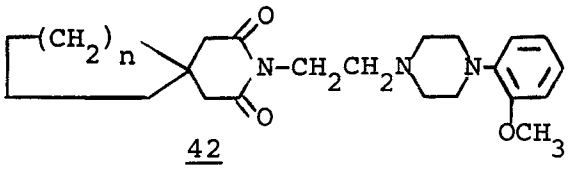

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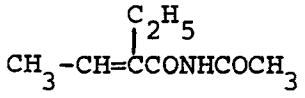

43

Compound 38 was one of the most active as a CNS depressant in mice of a series of substituted benzamides. Hypotensive properties paralleled the depressant action.[89] The quinazolone 39 (SL 146) was reported to have a pharmacological profile qualitatively similar to chloridazepoxide.[90] Initial pharmacology on Hoe 36,801 (40) indicates that the benzoxazine derivative has tranquilizing properties with a stimulant component.[91] A series of phenylisoquinolines and the corresponding dihydro and tetrahydro derivatives were prepared. Preliminary pharmacological screening indicated that these compounds had CNS depressant properties.[92] A series of pyrazino[1,2-a]quinolines exhibited a variety of pharmacological effects, dependent on the substituent pattern, including antireserpine, depressant, hypotensive effects.[93] Racemic glaziovine (41) was shown in pharmacological studies to be similar to chlorpromazine. However, unlike chlorpromazine, no dose response was noted. In some respects (±) glaziovine appeared to act like a minor tranquilizer.[94]

A series of β-aminopropionohydroxamic acids and β-aminopropionic esters showed an initial CNS stimulation followed by depression in rats. Almost all of the compounds reported exhibited hypotensive properties in cats.[95]

A series of N-piperazinyl-alkyl substituted cyclic imides related to 42 were prepared and screened for psychosedative activity. Preliminary pharmacology indicates that these compounds possess in varying degrees, psychotropic properties typical of the major tranquilizers. Structure activity relationships are discussed.[96]

A series of nineteen 5-spiroalkane and 5-spiro-4-piperidine derivatives of 2-amino-2-oxazoline-4-one were prepared. Three of these compounds showed characteristics of CNS stimulants, while the rest were weak CNS depressants.[97] Clinical evidence for the efficacy of homeostan (43) as a minor tranquilizer has been reported.[98]

Substance P, a polypeptide of molecular weight of about 1650 has been found in various parts of the anatomy. This compound exhibits CNS depressant effects and is proposed as a transmitter substance in sensory pathways.[99]

Pharmacological Investigations - Several additional studies on the role of the biogenic amines in the causes and treatment of mental disorders have appeared this year.[100, 101, 102, 103] Weischer and Opitz[104] have suggested that lithium chloride alters the metabolism of monoamines in the brain. A summary of The Chemistry of Psychotropic Drugs in 1969 was reported by Protiva.[105]

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Chapter 2. Antidepressives and Stimulants

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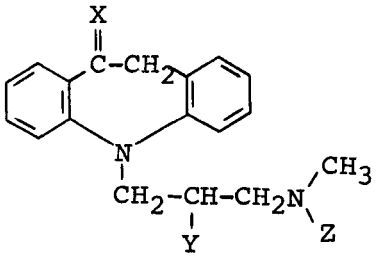
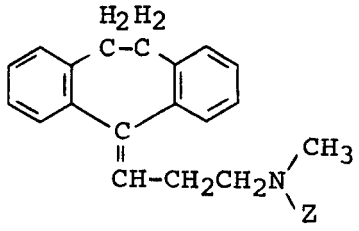
I. ANTIDEPRESSIVES

New Data and Structures - In a preliminary study with depressed outpatients, imipramine (1) and ketipramine (2) were approximately equivalent in therapeutic effectiveness.[1] A comparison of desipramine (3) with a placebo in both endogenous and neurotic depression showed desipramine to be superior to placebo in patients with endogenous depression after 5 days, but no significant difference in response was noted in the neurotic patients.[2] Further chemical modification of imipramine yielded a new derivative, Leo 640 (4), a compound having a pharmacological profile similar to that of imipramine, but considerably less toxic. Preliminary pharmacological studies of this material in man gave encouraging results.[3] Trimepramine (5), an imipramine analog with sedative properties, was slightly less effective than amitriptyline (6) in the treatment of neurotic depressed outpatients.[4] No significant difference was observed between imipramine and nortriptyline (7) used in the treatment of depressed female outpatients.[5] A liquid oral form of imipramine (pamoic acid salt) was more effective than a placebo in the treatment of non-schizophrenic and post-alcoholic depression.[6,7]





1, X = H_2; Y = H; Z = CH_3

2, X = O; Y = H; Z = CH_3

3, X = H_2; Y = H; Z = H

4, X = H_2; Y = H; Z = $CH_2C(=O)$-C$_6$H$_4$-Cl

5, X = H_2; Y = CH_3; Z = CH_3

6, Z = CH_3

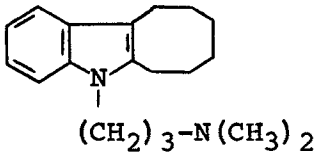
7, Z = H

The absorption of tricyclics varies considerably in patients. Significant differences were observed in the plasma levels of nortriptyline (7) in individuals receiving the same amount of drug; however, there was a positive correlation between the plasma levels and the subjective side effects. The monitoring of plasma levels of a drug might be rewarding in terms of improved patient care.[8]

Further clinical studies of iprindole (8) showed that the time of onset of the therapeutic response was similar to that for other tricyclics, but iprindole produced fewer troublesome side effects.[9-12] The question of heptatotoxicity related to iprindole therapy has been discussed.[13-15] A single dose of iprindole enhanced and prolonged the psychomotor activity elicited by D-amphetamine in the rat, probably due to an inhibition of the metabolism of D-amphetamine.[16] A series of epoxy compounds related to amitriptyline was highly active in reversing tetrabenazine-induced depression in mice; MK 940 (9) was about 80 times more active than amitriptyline in this test procedure.[17] In a preliminary clinical study, depressed patients receiving daily doses of 8-24 mg (9) were found to be more stimulated than were patients receiving 150-mg daily doses of amitriptyline. Both compounds exerted a greater antidepressive effect than did a placebo. A double-blind comparison of (9) with an established antidepressive having some stimulatory effect, such as imipramine, in an acutely depressed population was recommended.[18] Although the structure of azaphen (10) is markedly different from that of the usual tricyclics, its pharmacological profile is similar. Azaphen exhibits sedative properties and is used clinically in the USSR for the treatment of depression.[19] A narcotic antagonist, cyclazocine (11) showed clinical and EEG patterns similar to those produced by the tricyclic antidepressives. An improvement was observed when depressed patients were treated with cyclazocine; however, secondary effects were common, indicating a narrow therapeutic range for this agent.[20] A clinical study of four different nontricyclic structures, each having at least one pharmacological property considered useful for predicting antidepressive properties in man, showed a poor correlation between the results of animal testing and subsequent clinical effects.[21]

A series of partially hydrogenated analogs of amitriptyline and related tricyclics was prepared; the most active, (12), was slightly more potent than amitriptyline in antagonizing the central effects of Ro 4-1284 in rats.[22] The pyrrolidino com-

pound, AHR-1118 (13), exhibits a pharmacological and neuropharmacological profile in animals similar to that of the tricyclics and appears to merit study in man.[23] The amido compound, N-1157 (14), antagonized reserpine-induced toxicity and hypothermia in rats, and potentiated reserpine-induced hypermotility in mice.[24]



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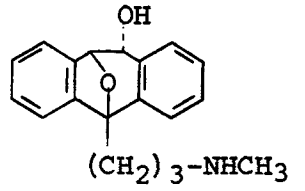

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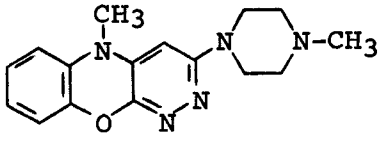

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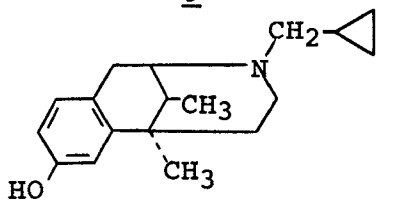

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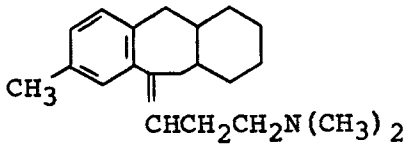

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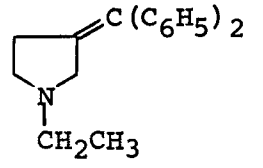

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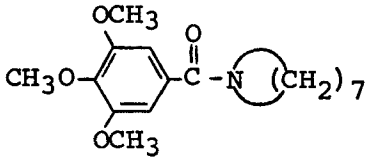

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The speed and efficacy of imipramine in the treatment of depression were enhanced by the addition of thyroid hormone.[25, 26] This combination was effective in patients who became resistant to the tricyclic antidepressives.[27] An increasing number of studies utilizing a combination of a standard tricyclic antidepressive and a small quantity of a tranquilizer has been reported.[28-35]

Small quantities of CNS depressants enhance the spontaneous increase in motor activity caused by D-amphetamine. This increase in motor activity may be due to an inhibition of fear and anxiety, thus leading to a smooth and uniform increase in activity.[36]

Cardiovascular side effects continue to be reported following therapy with the tricyclic antidepressives.[37-39] The tricyclics antagonize the hypotensive effects of guanethidine.[40] A patient who responded well to phenelzine, an MAO inhibitor, experienced a crisis after ingesting a meal of beef liver, which was subsequently found to have a high p-tyramine content.[41] Hypertensive crises associated with the administration of MAO inhibitors have diminished the utility of these agents in the treatment of depression. A comprehensive review on this subject has been published.[42] An updated review of the cardiac toxicity of psychotropic drugs has been published.[43] A study of the relation of sex and aging to levels of amines and monoamine oxidase in human brain, plasma and platelets showed that MAO activity in these three sources of enzymes was higher in women than in men of the same age, and that these MAO levels tended to rise after the age of 40. This finding supports the known higher incidence of depression in women and in the elderly of both sexes.[44]

Testing Procedures - The effects of a variety of tricyclics on the grooming behavior of reserpinized white mice were reported. This test procedure may be a useful tool in the further classification of the effects of antidepressives on the central nervous system.[45] The induction of aggressive behavior in newly hatched chicks might serve as a useful test in the evaluation of new antidepressive drugs.[46] Studies on central 5-hydroxytryptamine neurotransmission in the rat showed that imipramine potentiates the effects of tryptophan, indicating that a combination of tryptophan and imipramine might be more effective than either alone in the treatment of depression.[47] A preliminary clinical paper reported that high doses of L-tryptophan produced an antidepressive response similar to that elicited by imipramine.[48] Imipramine caused a decrease in the rate of disappearance of norepinephrine from the rat brain after a single intraperitoneal dose, but not after long-term administration by this route. This result may explain the delayed onset of action of the tricyclic antidepressives, and suggests that thyroid hormone or pharmacological agents that increase the turnover of norepinephrine in the brain, if

administered in combination with the tricyclics, may accelerate and enhance the clinical antidepressive effects of the latter.[49] A study of female patients gave evidence implicating cyclic AMP in depression and mania. A distinct decrease in the levels of cyclic AMP in the urine was observed in various stages of depression, but a marked increase of these levels was noted in the state of mania. In addition, imipramine, amitriptyline, nortriptyline and protriptyline were eight times more potent than caffeine as competitive inhibitors of rat-brain cyclic AMP phosphodiesterase. Cyclic AMP was also effective in reversing reserpine-induced depression in mice.[50] Experiments in vitro indicated that the antidepressives inhibit cyclic AMP phosphodiesterase. This inhibition would tend to increase the low level of cyclic AMP in the depressed patient.[51]

Clinical Reviews - A chapter dealing with differential drug effects in seven categories of depression has been published.[52] The present status of antidepressive therapy is also reviewed.[53-58] The effect of drugs that alter brain amines on the switch process from depression to mania was studied.[59] Publications dealing with the treatment of mania with lithium salts are increasing at a rapid rate. A critique of these studies indicates that the available data demonstrate neither efficacy nor inefficacy, primarily because of inadequate experimental design.[60] Serious side effects have been observed during therapy with lithium carbonate.[61]

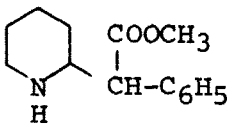
II. CENTRAL STIMULANTS

Methylphenidate (15) and magnesium pemoline (16) were more effective than placebo in mildly depressed outpatients; however, psychiatric patients did not respond well to these stimulants.[62] In a study dealing with a group of "neurophysiologic immature" inpatient children, minimal doses of methylphenidate produced favorable improvement in five of eight children.[63]

A clinical study of prolintane (17), a compound related to amphetamine (18), was conducted in fatigued volunteers. Prolintane (40 mg) was less effective than D-amphetamine (20 mg) in producing definite stimulant, euphoriant, anorectic and sympathomimetic clinical effects.[64] A preliminary study of the stimulant, pyrovalerone (19), indicated a favorable effect in the management of emotional symptoms associated with menopause.[65]

The piperidino derivative, SU-19789B (20), produced a stimulant effect in mice and rats similar to that of methylphenidate; however, (20) did not change the duration of sleep induced by hexobarbital in mice (methylphenidate prolonged such sleep). Neurophysiological studies in the cat suggested a peripheral site of action for this compound.[66]

Of a series of substituted oxazolines related to pemoline, (21) was the most active stimulant in mice (about 1/10 as active as D-amphetamine).[67]



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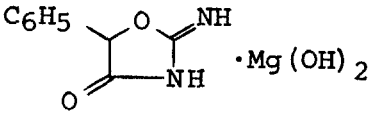

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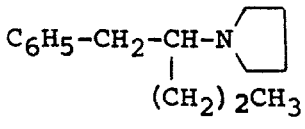

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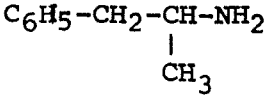

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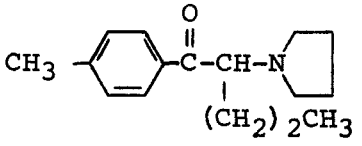

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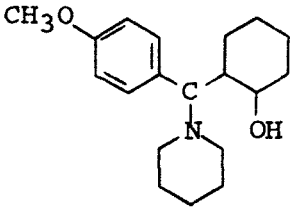

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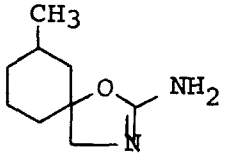

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Studies on the inhibition of uptake of norepinephrine by the adrenergic nerves in the rabbit aorta showed a correlation between biological activity and the structural conformation of a series of stimulant compounds.[68]

Amphetamine inhibited the oxidative deamination of norepinephrine in rabbit-brain cortex, suggesting that the mechanism of action of amphetamine is primarily an inhibition

of the uptake of the norepinephrine into the neuron, thus limiting access of norepinephrine to intraneuronal monoamine oxidase.[69] A study comparing the D- and L-isomers of amphetamine and their inhibition of norepinephrine and dopamine uptake in rat brain also suggests that the inhibition of norepinephrine uptake is the major mechanism of action of D-amphetamine.[70] A comprehensive review of stimulants has been published recently (see J. H. Biel, in "Amphetamines and Related Compounds," E. Costa and S. Garattini, Eds., Raven Press, New York, 1970, pp. 3-19).

SUMMARY - The enhancement of the speed and efficacy of imipramine by the addition of thyroid hormone to the treatment program represents a promising development in the control of depression. The apparent clinical failure of many new structures that exhibited high activity in one or more animal test procedures is indicative of the poor correlation between animal models and the complex nature of human depression. Biochemical, neuropharmacological and human pharmacological studies may yield new clues to the discovery of more effective, rapid-acting, and less toxic antidepressives than those that are now available.

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Chapter 3. Hallucinogens

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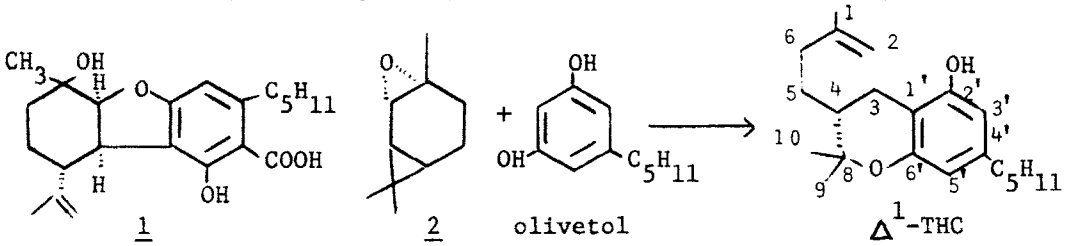
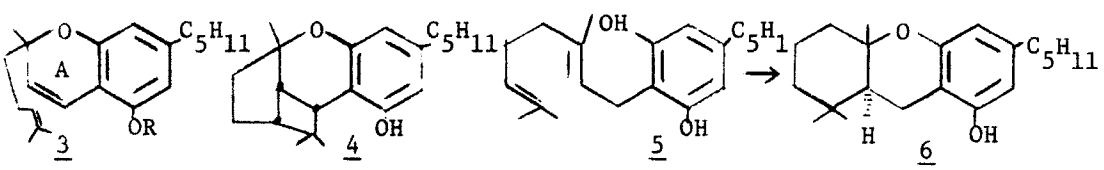
During 1970, the momentum gathered last year in the development of the chemistry and biology of marihuana and ergot alkaloids has continued and much more clinical data has appeared which gives a clearer definition and evaluation of the action of various hallucinogens in man. Reviews on hallucinatory substances from plants,[1] on psychopharmacology[2] including studies of hallucinogens in man and animals and on the mechanism of action of hallucinogenic drugs on a possible serotonin receptor in the brain,[3] have appeared. Papers on the various aspects of hallucinogens including a theory of hallucinosis, which were presented at a psychotomimetic drugs workshop in 1969 were published in book form.[4]

Chemistry - Rapid advances continue to be made in the chemistry of cannabinoids. Various papers on the botany, chemistry and pharmacology of marihuana have appeared in book[5] form and are already out of date. A more up-to-date version is given in a review[6] article. A taxonomical classification of Cannabis Sativa has been reviewed.[7] Contrary to the general view, that the active constituents of marihuana occur in the female plant only, both male and female flowering tops show similar THC content.[8] In addition, it has been reported that the cannabidiol content is high and Δ^1 and $\Delta^{1(6)}$-THC content is low in hemp cultigens while the reverse appears to be the case in plants grown for smoking.[8,9] Induction of female flowers on male plants of C. Sativa by 2-chloroethanephosphonic acid has been reported.[10] A n-propyl analog[11] of Δ^1-THC and cannabielsoic acid A[12] (1), a new type of cannabinoid, have been isolated from hashish. The latter has been synthesized by a novel photo-oxidative cyclization of cannabidiolic acid. A stereospecific synthesis of (-)-Δ^1- and (-)-$\Delta^{1(6)}$-THCs from (+)-trans-2-carene oxide (2) has appeared. This provides an entry into cannabinoids via carane derivatives and is the first one step stereospecific synthesis of Δ^1-THC.[13] The revised structure of cannabicyclol (4) has been confirmed by an X-ray study.[14] Various stereospecific cyclizations

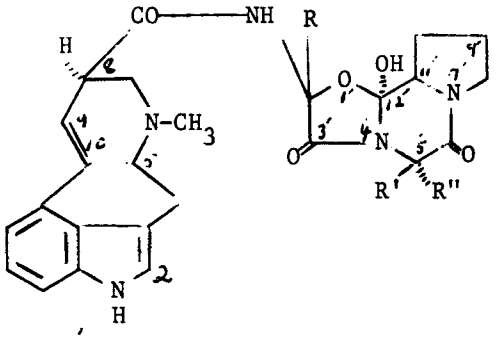




and isomerizations of cannabichromene (3, R=H) have been reported.[15] It is converted to 4 with BF_3 whereas its acetate (3, R=$COCH_3$) gives the acetate of Δ^1-3,4-cis-THC. The latter contradicts the postulation[16] that in this series, once the chromene ring A is formed, it remains. Highly stereoselective cyclizations of cannabinoid 1,5 dienes[17] e.g., cannabigerol (5) → 6 were reported. The suggestion has been made that these reactions may represent an organic model for related biochemical cyclizations.

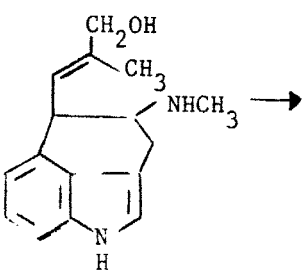
The results of a conformational analysis of Δ^1-THC and related compounds based on Westheimer and extended Huckel M.O. calculations, are in substantial agreement with PMR observations resulting from Overhauser and Solvent effect studies.[18] Detection methods for marihuana constituents have been developed.[19,20]

Alkaloids of the ergoxine group, i.e., ergonine [7a, R"=$CH(CH_3)_2$] and ergoptine [7a, R"=$CH_2CH(CH_3)_2$] were synthesized by the method previously used for related ergot alkaloids.[21] The synthesis of 2'β-iso-



7 a, R = CH_2CH_3, R' = H
b, R = $CH(CH_3)_2$, R' = H, R" = $CH_2CH_2CH_3$
c, R = $CH(CH_3)_2$, R' = H

propyl-5α-propyl-9,10-dihydro ergopeptine (7b, 9,10-dihydro) and its N'-methyl derivative was described.[22] Numerous derivatives of this class of compounds were synthesized and claimed to be useful in the treatment of migrane and hypotension.[23] The bromo derivative of α-ergocryptine [7c, R"=$CH_2CH(CH_3)_2$] was found to be much less toxic in its emetic and vascular effects compared to the parent compound. It inhibits the secretion of prolactin, fertility and lactation[24] and showed antitumor activity[25] (3-6 mg/kg s.c.). Ergocornine [7c, R"=$CH(CH_3)_2$] and its methanesulfonate (0.2 mg s.c.) also showed antitumor activity in rats[26] and mice[27] respectively. Biosynthetic studies relating to the origin of the side chain of ergometrine,[28] the proline part of the peptide chain of ergotoxine[29] and the amide substituent of N-(α-hydroxyethyl) lysergamide were carried out in a culture of Claviceps paspali.[30] A review on ergot alkaloid fermentations has appeared.[31] Enzymatic oxidative closure of Ring D of the ergolene nucleus of chanoclavine 8 in vitro gave elymoclavine 9 without the intermediacy of agroclavine 10. An intermediate 11 has been postulated.[32] The synthesis and incorporation of chanoclavine aldehyde[33] into 9 by claviceps was also shown. On the other hand in the dihydrochanoclavine series it is suggested that the ring closure involves the $-CH_2OH$ group.[34] Central stimulatory amphetamine-like effects were found in lysergic acid



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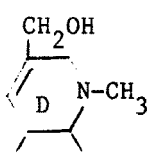

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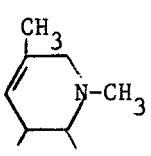

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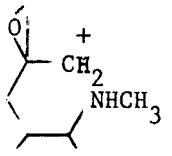

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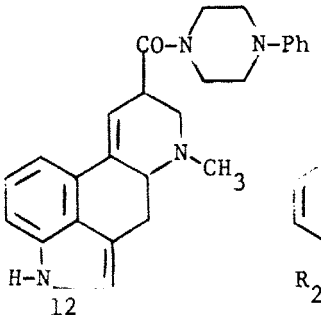

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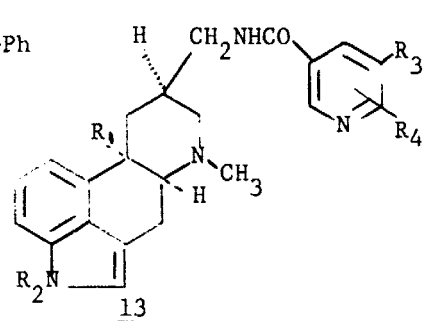

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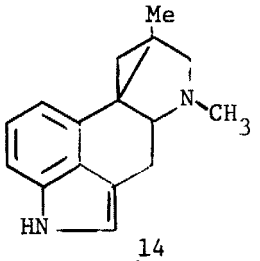

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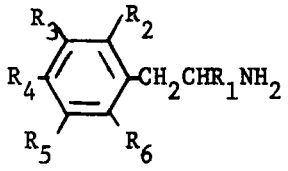
piperazides 12.[35] Various ergoline derivatives of type 13 (R_1=H,OCH_3; R_2=H,CH_3; R_3=H,Br,Cl,F and R_4=H,6-Cl, 2-Cl) were made and showed adrenolytic and hypotensive properties.[36] A long lasting hypotensive effect (0.3 mg/kg) was also claimed for D-6-methyl-8-(2-hydroxyethyl) ergoline.[37] A new alkaloid cycloclavine,[38] (14) containing a three-membered ring was isolated from Ipomoea hildebrandtii. A novel procedure for the determination of dissociation constants of ergot alkaloids was reported.[39]

The photolysis of N-chloroacetyl mescaline[40] has provided some additional examples of unusual rearrangements of the mescaline skeleton. Numerous new mescalinoids[41] of type 15 were synthesized (where R_1=H,CH_3; R_2 and R_5=H,Cl,OCH_3; R_3 and R_6=H,Cl and R_4=various ethers) as potential psychotropic agents. In a study of hallucinogenic amphetamines, a significant correlation was found between the ease of perturbability of the amphetamine π electrons and their activity. However, it did not account for the activity of all the compounds.[42]

A versatile route to iboga and vinca alkaloids has been developed.[43] The sequence utilizes in its penultimate step a reductive cleavage reaction to generate the nine-membered ring system of the cleavamine molecule. In this way carbomethoxydihydrocleavamine (16) is synthesized which is then converted to dihydrocatharanthine (17b, R=$COOCH_3$) and coronaridine (17a, R=$COOCH_3$). The latter is then converted to ibogamine (17a, R=H). Synthesis of ibogamine[44,45] and analogs[46,47] were also reported, some of which showed cardiac activity. Psilocybin was isolated from P. Subaeruginosa[48] but no psilocin was detected. A synthesis of psilocin[49] and its analogs was reported utilizing a new synthesis of the indole nucleus. The chemistry of Amanita muscaria components has been reviewed[50]



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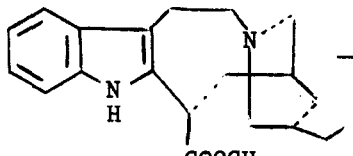

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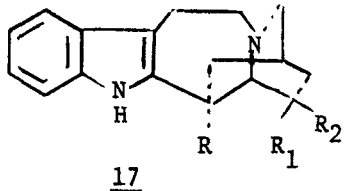

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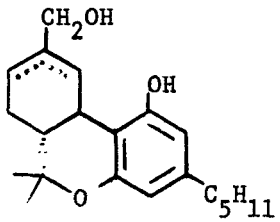
in detail and the pharmacology of ibotenic acid and muscimol are given. Isolation of (-)-allomuscarine is reported.[51] Dihydrokawain-5-ol,[52] a new alcohol and two new pyrrolidines[53] have been isolated from P. methysticum. Reactions of (±)kawain, methysticin and yangonin with acids were described.[54]

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

Biology - Interest has centered around metabolic studies of Δ^1- and $\Delta^{1(6)}$-THCs. Liver homogenate or its supernatant metabolized Δ^1-THC to the pharmacologically active 7-hydroxy-Δ^1-THC[55] (18). An inactive metabolite, 6,7-dihydroxy-Δ^1-THC was also identified. Similarly $\Delta^{1(6)}$-THC was shown to be metabolized to the active 7-hydroxy-$\Delta^{1(6)}$-THC both in vitro and in vivo.[56] Distribution of tritiated Δ^1-THC in the rat after inhalation of the smoke indicated that the main route of elimination was through the feces.[57] Studies on the disposition and metabolism of Δ^1-THC in man were reported.[58] Δ^1-THC-^{3}H binds to human plasma protein in vitro[59] and this may account for the slow elimination of the drug in man and animals. Δ^1-THC (4 and 16 mg/-kg) was found to potentiate the effects of pentobarbital (10 mg/kg) and prolong pentobarbital (30 mg/kg) sleeping time.[60] It causes tolerance to the behavioral effects in pigeons under a multiple schedule of food presentation. The dose (1.8 mg/kg) was gradually increased to 20 times its original value (36 mg/kg) without disrupting behavior and no withdrawal syndrome was detected when the drug was withdrawn.[61] Evidence of tolerance was also reported in mice.[62] Two heterocyclic analogs [19 and 20, R=$CH(CH_3)CH(CH_3)$-C_5H_{11}] showed qualitatively similar effects to Δ^1- and $\Delta^{1(6)}$-THC in un-



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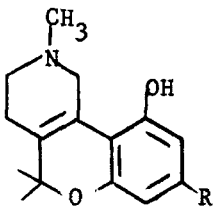

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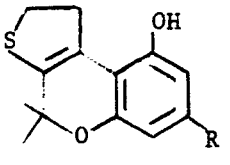

20

anaesthetized dogs (0.2-0.5 mg/kg i.v.). They potentiated epinephrine and norepinephrine in anaesthetized dogs (0.1 mg/kg) but did not show any reduction in blood pressure.[62] A water-soluble derivative (the δ-diethylaminobutyric ester) of Δ^1-THC[63] was reported in preliminary tests to have a pharmacological profile similar to Δ^1-THC. In a comparison of various CNS effects, the Gayer test[64] was found to be specific for picking out Δ^1-THC. A proteolytic enzyme edestinase[65] was isolated from the seeds of C. Sativa.

Research continues apace in LSD. In several mammalian species including man repeated administration of LSD leads to a regular cyclic response the amplitude of which seemed to be dose dependent and the period species dependent.[66] LSD reduces both the synthesis and turnover of 5-HT in mouse brain[67] and increases catecholamines[68] in the reticular formation of the mid-brain and decreases the levels in the hypothalamus. On the basis of a study of the flexor reflex and stepping reflex in chronic spinal dogs,[69] it was suggested that the mode of action of LSD-like psychotogens is similar to tryptamine but is different from that of 5-HT, serotonin. Like serotonin, however, LSD causes an increase in glycolysis in the liver fluke F. hepatica and an increase in the activity of phosphofructokinase.[70] A possible neuronal basis for the action of LSD has been proposed.[71] The behavioral effects of LSD and mescaline were markedly reduced in rats given p-chlorophenylalanine thus indicating serotoninergic mechanisms.[72] LSD affected the nest-building behavior[73] in mice (100 µg/-kg i.p.) and the performance of rats in the maze test.[74] The open field performance in rats[75] has been studied. It was suggested that genetic effects may play a role in determining the response to LSD in an electroshock alley T-maze test in mice.[76] 100R of X-irradiation produced the same amount of chromosome damage as 4.2 µg/ml LSD and the damage was linearly related to concentration.[77] LSD was reported to be teratogenic in mice[78] but different results were shown in other papers.[79,80] The effect of ergotamine on norepinephrine, epinephrine and isoproterenol in the dog was reported.[81,82] The synapse[83] appears to be the site of action of mescaline as indicated by subcellular uptake studies. It was also shown that the bradycardia and arrhythmia produced by mescaline in mice is CNS mediated.[84] Unlike LSD N,N-Diethyl-1,2,5,6-tetrahydro-1-methylnicotinamide markedly potentiated the behavioral effects of mescaline.[85] Differences were found in the effects of psychotogens on single midbrain raphe neurons.[86]

A detailed quantitative study of harmine metabolism in man and rats was reported (0.5 mg/kg i.v.). Harmol sulfate was the primary conjugate in rats and harmol glucuronide excretion predominated in man.[87] Harmine (i.v.) in cats, rats and humans induced bradycardia and hypotension and the effects of acetylcholine and epinephrine were potentiated due to inhibition of cholinesterase and MAO respectively.[88] The effect of harmine on serotonin-^{14}C metabolism was reported.[89]

The EEG and behavioral effects (cats)[90] and autoradiographic study (monkeys)[91] of harmaline have been reported. Muscimol and ibotenic acid (A. muscaria) showed an increase in serotonin levels in the midbrain and hypothalamus perhaps due to reduced serotonin turnover.[92] Methysticin was claimed to be better than mephenesin against strychnine poisoning.[93] Indolealkylamines were shown to affect 5-HT metabolism in a manner similar to LSD.[94]

Clinical - Initial symptoms on smoking cigarettes containing Δ^1-THC by 6 subjects were numbness and tingling of the extremeties, lightheadedness, loss of concentration, palpitation, sweating, tremulousness and weakness followed by increased mental impairment, mental confusion, loss of time sense and feeling of euphoria. An increase of heart rate by ~20 beats/-

min and a reddening of the conjunctiva was observed in 5 out of 6 subjects. The isomeric Δ^3-compound and its homolog synhexyl produced similar but less severe effects.[95] In another study where Δ^1-THC and synhexyl were administered orally, it was found that plasma cortisol and platelet serotonin were unchanged. The lack of major effects of marihuana-like drugs on these and other clinical measurements of stress corroborates the clinical observation that the drugs of this type are less stressful than the usual psychotomimetics.[96] High oral doses of THC (20-60 mg) induce temporal disintegration stemming partly from impaired immediate memory, disorganized speech and thinking.[97] In a laboratory setting both marihuana and alcohol seemed to be mild intoxicants. With THC the EEG changes were characterized by an increased abundance of low voltage fast (20-30 Hz) activity, decreased alpha abundance and slight alpha slowing. Interestingly the subjects who were regular users of marihuana were unable to distinguish between smoked marihuana and the THC free placebo.[98] A measured dose of Δ^1-THC (5 mg) by smoking, showed a significant decrement in motor performance tests in experienced subjects.[98a] A fatal intoxication by man due to cannabis smoking was reported. A toxicological study carried out 5 days after death showed only cannabinol in urine.[99] An article on adverse reactions to marihuana and suggested treatment has appeared.[100]

A study of the effects of LSD on human pregnancy was published.[101] The effect of LSD (1.5 µg/kg) on sleep-deprived men has been reported.[102] LSD (1.5-2 µg/kg) and mescaline (4-6 mg/kg) when administered orally produced an antidiuretic effect[103] in 10 of 16 subjects.

Quantitative EEG and behavior changes after LSD and Ditran (i.v.) were reported in a group of chronic schizophrenic patients.[104] Close correlations between EEG changes and alterations in psychopathology were seen after both the drugs. Compared to dimethoxyphenethylamine (DMPEA) its N-acetyl derivative (NA DMPEA) is more potent in rats. In a modified rising dose tolerance test (1.3-16.4 mg/kg) it did not show any hallucinogenic effect.[105] Proceedings of a symposium on amphetamines have been published.[105a] A comparison between N,N-dipropyl,N,N-diethyl and 6-flouro-diethyltryptamines was reported.[106] Following the administration of LSD or N,N-dipropyltryptamine in 36 subjects no increase in the activity of the two enzymes CPK (creatine phosphokinase) and aldolase was found. The results are discussed with reference to the known increase in the activity of these enzymes in acute psychoses.[107]

Ergotamine and ergometrine were shown to have potent venoconstrictor action in the forearm of normal subjects. It is suggested that this may be important in provoking the harmful cardiovascular side effects that are sometimes observed in patients with preexisting heart disease.[108]

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Chapter 4. Analgesics

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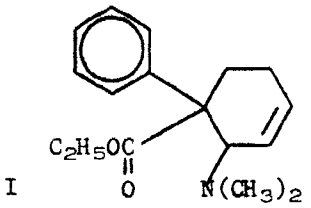
Research on strong analgesics has shown a relative increase in detailed structure-activity studies and investigations of biochemical and pharmacological mechanisms. Few new candidates have been proposed as non-narcotic replacements for morphine, and none appears to challenge the growing use of pentazocine.

In the area of mild analgesics some novel structures have been reported to have higher analgesic potency and fewer side effects than the compounds now in use.

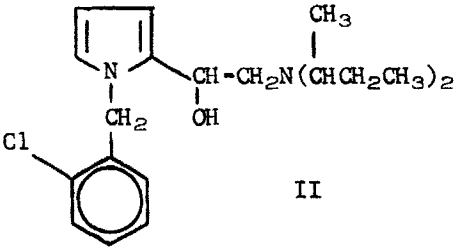
Reviews of recent work on analgesics[1] and on pain mechanisms[2] have appeared.

I. Strong Analgesics

A. New Clinical Studies - Only two of the newer compounds reaching clinical trial appear to be potentially useful as non-narcotic analgesics.



I



II

Tilidine (I) has been studied in over 3000 patients[3] and was said to be an orally effective agent nearly as potent as meperidine. Although definitive dependence studies in man have not been reported, extensive pharmacological and toxicological studies show little capacity to produce dependence in animals.[4]

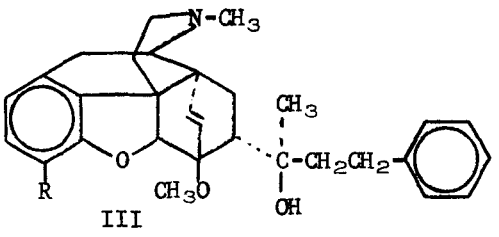
Continuing clinical studies of diviminol (II) have shown a favorable comparison with codeine in potency and lack of side effects.[5] No tolerance development or narcotic side effects were observed.

B. Pentazocine - Reports too numerous to list continue to appear. It has been demonstrated that pentazocine has properties of both morphine and nalorphine and can cause physical dependence.[6] However reports of abuse are relatively rare,[7] and narcotic control has not been recommended.

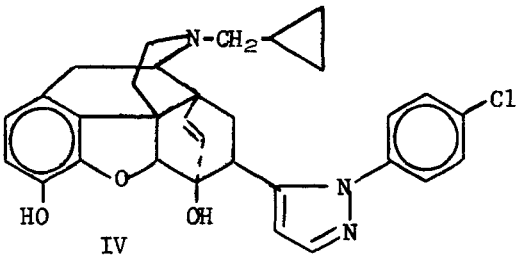
C. Structure-Activity Studies - Only work which includes new relationships or novel structures is discussed below. Unless specified otherwise, potency estimates refer to the mouse hot plate test and dependence capacities to morphine dependent monkeys.

1. Morphine - Originally B/C trans-morphine was said to be inactive, but it is now reported to be 10% as active as morphine. In this series, potency is not reduced by methylation of the phenolic hydroxyl,[8] and trans-isocodeine shows twice the potency of trans-isomorphine.

A negative effect of the hydroxyl was seen in the etheno-thebaine analog III--R=H which was more active (2000 times morphine) than the oripavine analog III--R=OH (1600 times morphine). This effect was not seen when the phenethyl group was replaced by alkyl.[9] The pyrazole analog IV was a very potent analgesic which showed no antagonism or support of morphine dependence.[10]

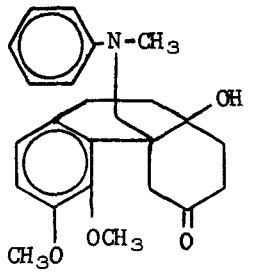


III

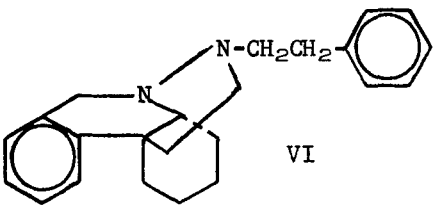


IV

The high analgesic activity (2.8 times morphine) of the substituted formal morphanone cleavage product V in the Haffner test appears to be due to the presence of the hydroxyl group.[11]



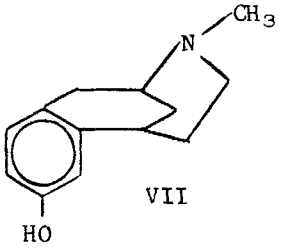
V



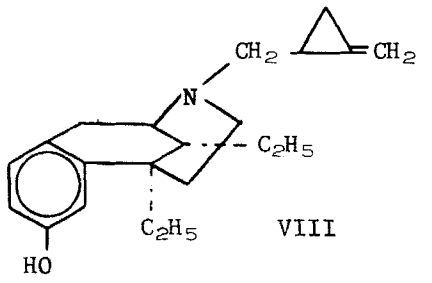
VI

In continued studies of azamorphinans,[12] VI was found to be half as potent as morphine and would not support morphine dependence.

2. Benzomorphans - The discovery that strong analgesic activity and narcotic antagonism are found in the levo forms of benzomorphans and weak analgesic activity and physical dependence capacity in the dextro forms has proved to have numerous exceptions. Both optical isomers of the analog VII, lacking a quaternary carbon atom, were analgesics with no physical dependence capacity and both showed antagonist properties.[13] Both levo isomers of VIII were potent analgesics which supported morphine dependence while the dextro isomers did not. None was an antagonist.[10]

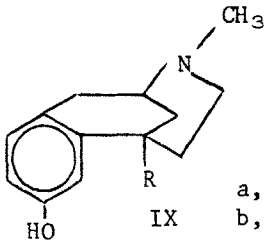


VII

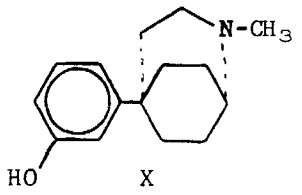


VIII

The benzomorphan-prodine hybrid IXa was more like prodine than a typical benzomorphan.[14] It would support morphine dependence in the monkey, in contrast to a previous benzomorphan-meperidine hybrid (IX-b). The stereoisomers of the phenylmorphan X behaved like benzomorphans rather than meperidine analogs.[13]

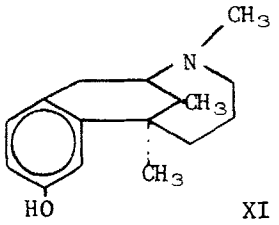


IX a, R=OCOCH3 b, R=COOC2H5

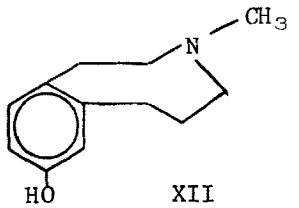


X

Contrary to most known analogies, expansion of the piperidine ring of metazocine gave the analogs XI with good analgesic activity.[15] Removal of the methylene bridge of VII to give XII destroyed activity.[16]

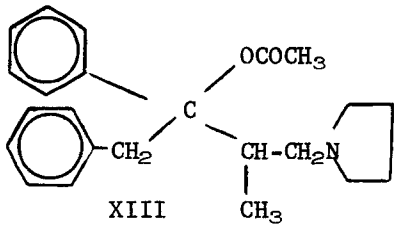


XI

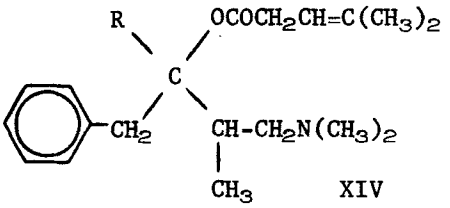


XII

3. Miscellaneous - Clinical studies of the close analog of propoxyphene XIII (α-d-form), Lilly 31518, showed analgesic potency about one-third that of morphine, and some problems of dependence were seen.[17] Three other analogs of propoxyphene XIV were active in the rat inflammed foot, but unlike propoxyphene not in the normal foot.[18]



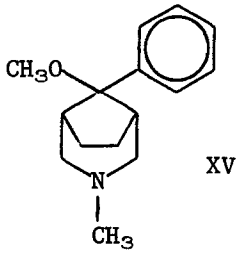
XIII



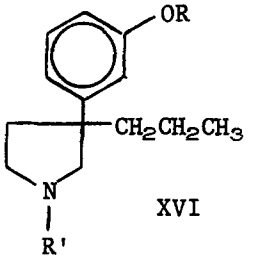
XIV

$R=C_6H_5CH_2$; C_2H_5; $-CH_2-CH=C(CH_3)_2$

Azabicyclane XV was reported to be 6-8 times as potent an analgesic as meperidine but to have much less anticholinergic activity. It was stated that tolerance developed rapidly but no dependence was seen.[19] In a series of 60 analogs (XVI) of profadol, maximum analgesic activity was found with an N-phenethyl group and a free phenolic hydroxyl.[20] Alkylation of the hydroxyl prevented the potentiating effect of the phenethyl substituent.

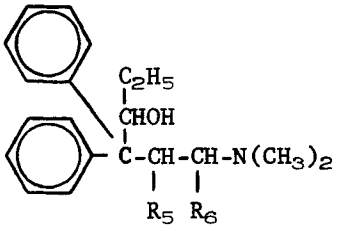


XV

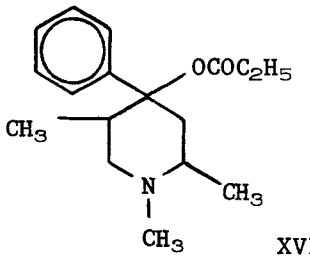


XVI

4. Conformational Studies - The absolute stereochemistries of the four isomethadol isomers (XVII, $R_5=CH_3$, $R_6=H$) have been deduced and compared with those of the methadols (XVII, $R_5=H$, $R_6=CH_3$). It was suggested that potency differences between enantiomers are due to an obstructive role of the methyl groups on receptor binding or intramolecular geometry. Stereochemistry of the isomers of trimeperidine (XVIII) was established.[22] A cis 5-methyl/4-phenyl is most advantageous for analgesic activity but orientation of the 2-methyl group has less influence.

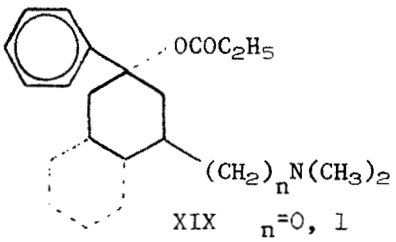


XVII

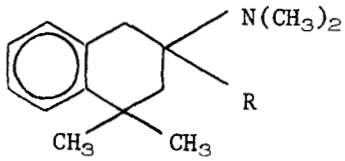


XVIII

A series of 8 compounds represented by the general formula XIX was synthesized to test the proposed requirement for an "out of plane two carbon chain" between the quaternary carbon and nitrogen in potent analgesics. None was a potent analgesic (>20% codeine), but the most active



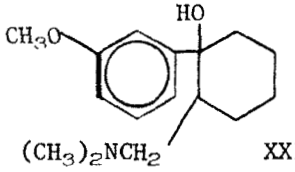
XIX n=0, 1



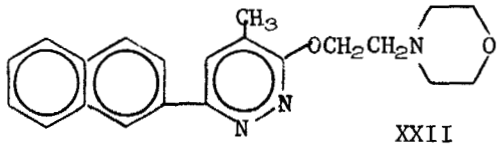
XX

were those with the greatest conformational flexibility. Synthesis of aminotetralins XX with 2-alkyl substituents to fix ring conformation[24] reduced analgesic activity below that of the parent (R=H).

D. New Compounds - Tramadol (XXI) was shown in the clinic[25] to have an analgesic effect of the order of codeine but CNS side effects were not tolerable. A series of pharmacological studies of XXII, Agr 614,[26] indicated that it had more potent analgesic activity and fewer side effects than other members of this large series.

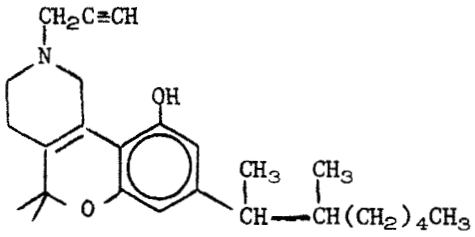


XXI

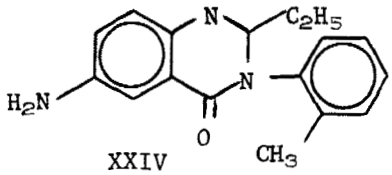


XXII

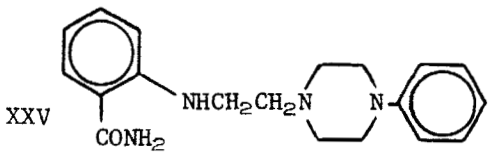
Compounds XXIII-XXV were reported[10] to have analgesic potencies between codeine and morphine and no capacity to support physical dependence. The cannabinol analog XXIII in morphine dependent monkeys produced a reaction resembling abstinence mixed with sedation. Significant analgesic activity of tetrahydrocannabinols in general has been hard to demonstrate.[27]



XXIII



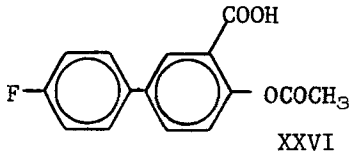
XXIV



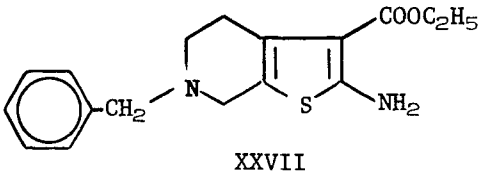
XXV

II. Antiinflammatory Analgesics

For complete coverage of this class of compounds refer to the chapter on Antiinflammatory Agents (Section 4). The compounds discussed below are those for which significant analgesic activity was reported.



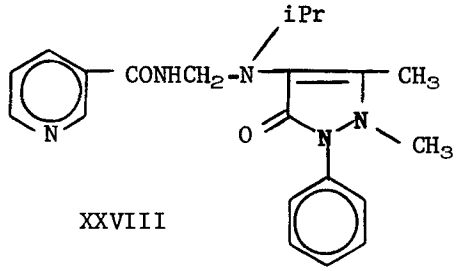
XXVI



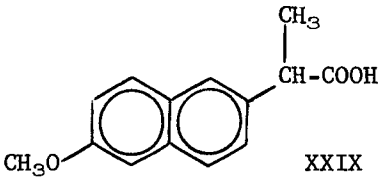
XXVII

Flufenisal, XXVI, in a clinical study on episiotomy pain[28] appeared about twice as potent as aspirin with longer duration of effect.

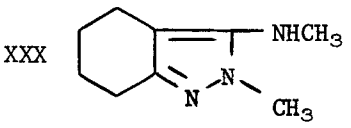
Analgesic activities of XXVII (Y-3642),[29] XXVIII (RA-101)[30] and XXIX (naproxen)[31] were reported to be superior to phenylbutazone in animal tests. Analgesic effects of XXX[32] and XXXI[33] were several fold greater than phenylbutazone without corresponding increases in anti-inflammatory activity.

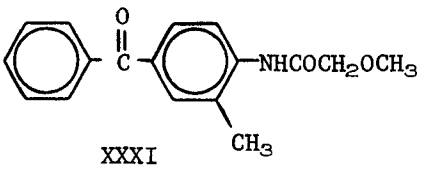


XXVIII



XXIX





XXXI

III. Biochemical Mechanisms

A great many reports of attempted correlation of levels of neurohumoral agents with analgesia and tolerance have appeared. In particular, the report that increased brain serotonin turnover was associated with tolerance and dependence, and that this could be prevented by p-chlorophenylalanine,[34] stimulated much investigation. However, conflicting reports[35],[36] still leave this question unresolved.

Many new data have supported a role for catecholamines in analgesia and the abstinence syndrome.[37],[38] Stimulation of brain catecholamine biosynthesis by morphine can be inhibited by narcotic antagonists and tolerance develops to this stimulatory effect.[39]

It was suggested that the apparent inhibition of brain protein synthesis by morphine is due to its effect on amino acid transport.[40]

Morphine has been shown to reduce cortical release of acetylcholine *in vivo* analogous to its *in vitro* effect.[41] Narcotic antagonists reverse this inhibition.

It was reported that TPN (3-methylsulfonyl-10-[2-(1-methyl-2-piperidyl)ethyl]phenothiazine) and chloropromazine inhibited development of tolerance to morphine as well as temporarily arresting the tolerance.[42]

In mice tolerant to levorphanol's effects, increased levels of drug in brain tissue were found, indicating lowered sensitivity of receptor sites.[43]

IV. Pharmacology

A mathematical model for analgesic potency has been constructed using the effective doses of drugs administered intraventricularly as a measure of intrinsic activity and distribution coefficients between heptane and water as a measure of transport into the CNS.[44] The results emphasize the importance of polarity for receptor activity and indicate passive transport into the brain.

Evidence for an active transport process for removal of morphine from cerebral ventricles was reported.[46] Inhibition by ouabain and potassium dependence suggest involvement of an ATPase.

Discussion of the extensive research on new pharmacological models for measurement of analgesia and addiction liability is outside the scope of this review.

V. Narcotic Antagonists

Some clinical studies have indicated the use of cyclazocine or naloxone may be an effective treatment for addiction.[48] More definitive studies are in progress.

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Chapter 5. Antiparkinsonism Drugs

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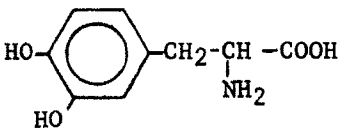
Physicians now can treat patients with parkinsonism more effectively and definitively. This improvement of therapeutic outlook has followed a spurt of recent research activity. This research and the drug developments stemming from it are summarized here.

The medicinal chemist should consult the recent review of Engelhardt and Stone[1]. Other reviews of pertinent area stress therapeutics (Klawans, Ilahi and Shenker[2]; Calne[3]), pharmacology (Hornykiewicz[4]), physiology (Shute and Lewis[5,6]) and anatomy (Carman[7]).

Levodopa - This naturally-occurring amino acid (I, L-3,4-dihydroxyphenylalanine) was first used to treat parkinsonian patients in 1961. Morgan



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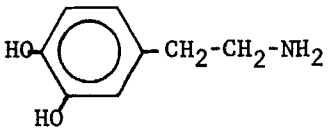


II

and Bianchine[8] and Barbeau[9] have well summarized the medical use and history of levodopa. A more detailed monograph with extensive documentation has recently appeared[10].

The background and theoretical basis of levodopa therapy can be summarized thus:

1. Parkinsonian patients' brains show cytological deterioration, principally but not exclusively in midbrain and basal ganglia.

2. Parkinsonian patients' brains show a deficiency of dopamine (II) in the substantia nigra of the midbrain and in the corpus striatum (caudate nucleus and putamen) in the basal ganglia of the forebrain.

3. The depletion of dopamine is presumably due to degeneration of fibers of the nigrostriatal tract.

4. Dopamine may act as a specific transmitter at certain "dopaminergic" synapses.

5. Dopamine cannot cross the blood-brain barrier, but its immediate biochemical precursor levodopa does so readily and could restore function by replenishing dopamine.

Although the results of early trials were conflicting, probably due to the use of low doses and brief treatment periods, Cotzias et al.[11,12] convincingly established the striking efficacy, of a degree exceeding that of all previous medications, when high sustained doses of levodopa were administered. Many others subsequently confirmed this finding. In over 3,000 patients the success rate has been between 70 and 80%.

Levodopa favorably altered nearly all symptoms of Parkinson's disease, though not equally. Rigidity and hypokinesia have responded well and early. Tremor responded irregularly and later. Many other symptoms were partially or completely reversed by levodopa, including impaired posture, loss of associated movements, increased sebum secretion, impaired speech and sialorrhea. Levodopa induced greater overall improvement in moderately to severely affected patients than did anticholinergic drugs. Most observers noted no loss of levodopa efficacy with time in contrast to previously effective drugs. Levodopa is superior to surgery, which is plagued by irregular response, serious hazards and recurrence of symptoms.

Levodopa works best in patients with mild disease of short duration but it also helps patients with severe longterm disease. While it helps all forms of the disease, postencephalitic cases are very sensitive to both the therapeutic effects and the side effects. Levodopa treatment must always be started with low doses (300 to 500 mg per day) and the dose must be slowly and carefully increased up to the point of optimal efficacy or limiting side effects (usually 2.5 to 6 g per day, although a few patients may receive the maximal recommended dose of 8 g). Skill and diligence in management of dosage, symptoms and side effects is essential to effective therapy with this drug, since there are daily and within -day variations in effect, which are related to the short half-life and to interactions with dietary amino acid intake (mainly phenylalanine) which cause refractory periods of rapid onset and variable length.

Side effects are numerous and troublesome occurring in nearly 100% of patients, but are not generally serious. They have caused termination of drug in about 5% of cases. They involve nearly all organ systems but the most important are 1) nausea, vomiting and anorexia, 2) cardiac dysrhythmias, 3) hypotension, 4) abnormal involuntary movements, and 5) behavioral and personality changes.

Nausea, often accompanied by vomiting, is seen at some stage of therapy in all patients. Loss of appetite may occur with or without these signs. Gastro-intestinal signs, despite their frequency, are rarely dose-limiting. Co-administration of food may control nausea. Phenothiazine anti-emetics and pyridoxine may block or reduce levodopa efficacy and should not be used for control of nausea or emesis.

The commonest cardiac problems with levodopa therapy are sinus tachycardia and premature ventricular contractions, with rare instances of atrial fibrillation. Cardiac deaths with ventricular tachycardia and fibrillation have been reported but drug causation is unclear. The cardiac side effects of levodopa can be a serious threat particularly to patients

with coronary artery disease. Levodopa therapy in these patients should be instituted cautiously in the hospital with access to monitoring and control equipment. Levodopa cardiac responses are mediated by conversion to dopamine, which activates β-adrenergic receptors. A β-adrenergic blocking agent such as propranolol would be logical therapy for cardiac problems when they occur.

Patients with Parkinson's disease have a lower blood pressure than age- and sex-matched controls and often show orthostatic hypotension. Contrary to expectations, levodopa produces increased orthostatic hypotension which may become clinically important. The incidence is probably higher than the 25 to 35% reported since one careful study of blood pressure showed 75% of patients with a decrease of greater than 10 mm Hg in blood pressure on standing. Dizziness, vertigo and syncope are uncommon. They occur early, can generally be controlled, and disappear later with the development of tolerance. The mechanism of the orthostatic hypotension due to administration of a pressor amine precursor is not clear and its occurrence is surprising.

Abnormal involuntary movements frequently accompany optimal improvement with levodopa. The duration and the dose are related to the occurrence of these movements so that up to 73% of patients have them after 12 months of treatment. Abnormalities are seen earliest in the head and mouth, then later in the limbs and trunk, sometimes becoming violent and severe. Usually they can be reversed by lowering the levodopa dose or by adding other drugs (phenothiazines, haloperidol or pyridoxine) but with both approaches some therapeutic benefit is often lost.

The parkinsonian population, generally aged, has a significant incidence of impairment of mental function (memory, judgment, dementia and social adaptation). Levodopa treatment is accompanied by behavioral and personality changes in some patients. The central stimulant effect seen early in therapy may be related to a toxic delirium which levodopa can cause. The more florid cases show paranoid ideas, delusions, hallucinations and loss of judgment, but frequently a milder picture is seen with less disruptive stimulant effect and nervousness, anxiety and sleeplessness (sometimes with vivid dreams) plus some autonomic signs. Rarely sedation, malaise, and emotional depression to the point of suicidal ideas are noted, but drug causation is unclear. Levodopa has been publicized as an aphrodisiac, but this may be mainly the talk of toxic delirium patients; however the remitted illness of a previously incapacitated victim may permit the appropriate renewal of sexual performance. There is not firm evidence that it has fulfilled the ancient concept of a specific aphrodisiac.

Many other infrequent symptoms and abnormal laboratory findings have been associated with levodopa treatment. Metabolites cause the urine to turn reddish and then black. Many drug interactions are possible; few are confirmed, but this possibility should be watched. Some monoamine oxidase inhibitors reported effective in Parkinson's disease caused hypertensive reactions in combination with levodopa, but tricyclic antidepressants

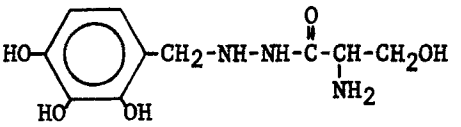
(imipramine and amitriptyline) also effective in parkinsonism, have been used with levodopa without serious side effects.

Levodopa was approved by FDA in June 1970 for marketing in the treatment of Parkinson's disease. Drug costs were relatively high but are declining and drug availability is increasing.

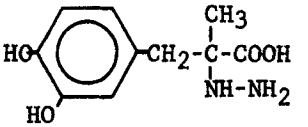
It is not known whether levodopa alters the steady progression of Parkinson's disease, since few patients have been observed longer than 3 years. Generally it is stated that no further deterioration has been observed in successfully-treated patients.

Other medical uses for levodopa include success in treating the akinetic-dystonic syndrome of manganese poisoning in miners. Other neurological and psychiatric indications are not yet established. Levodopa has yielded conflicting results in dystonia musculorum deformans, Wilson's disease, Huntington's chorea, and progressive supranuclear palsy. Reports of limited trials of levodopa for the possible treatment of depression[13-16] have appeared, but they are difficult to interpret. Studies to resolve the issue are in progress.

Decarboxylase Inhibitors - At least two agents designed to decrease the peripheral enzymatic inactivation of levodopa have been tried. Both Ro 4-4602 (III) and MK 485 (IV) have been reported to lower the optimal



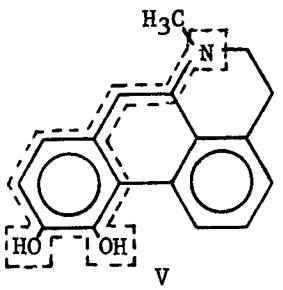
III



IV

levodopa dose to one-fifth to one-eighth. It is not yet clear that any therapeutic advantage is achieved in efficacy or greater side effect margin beyond the decrease in the levodopa dose required[17-21]. Abnormal involuntary movements are still seen at the lower dosage. More information is required to say whether or not this combination therapy with levodopa will be useful.

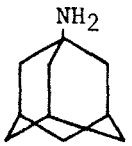
Apomorphine - This morphine derivative (V, common structural elements with dopamine, II, indicated) has recently assumed considerable theoretical and possible practical importance in understanding the pharmacological role of levodopa and dopamine in the therapy of parkinsonism. Schwab[22] reported that it relieved tremor in patients but was of too short duration for useful therapy and was only effective parenterally. Then, Vernier[23,24] found that apomorphine strikingly reduced the tremor of monkeys with midbrain lesions made by the technique of Ward[25], Peterson[26] and of Carpenter[27]. Poirier[28] and Goldstein[29] have used these methods to study brain catecholamine changes and to study these



V

and other drug effects. More recently Cotzias[30] has confirmed Schwab's finding in man. The work of several groups has now converged to indicate that apomorphine acts upon many physiological systems and vascular beds to cause effects similar to those of levodopa or dopamine[31,32]. Thus apomorphine probably directly activates "dopaminergic" receptors and its mode of action in parkinsonism is clearer. Sourkes[33] has suggested that levodopa or dopamine may act partially through metabolic conversion to aporphines resembling apomorphine.

Amantadine - Amantadine hydrochloride (VI) was introduced in 1967 as an antiviral agent for influenza[34,35]. In 1968 Schwab et al.[36] noted that amantadine caused remission of parkinsonian symptoms in a patient treated prophylactically during an influenza epidemic and they subsequently studied the effect in 163 patients. Since then more than 80 relevant papers have appeared of which more than 30 are reports of clinical studies. They include over 1,000 patients with over 300 from controlled trials (e.g.[37,38,39]). Most authors concluded that amantadine shows some degree of antiparkinsonian activity (e.g.[40,41,42]). The onset of effect is generally rapid, 24 to 48 hours, compared with weeks or months with levodopa. The clinical reports suggest that the amantadine degree of activity is at least as great and perhaps greater than that of the standard antiparkinsonian drugs, principally anticholinergics, but not as great as that of levodopa. Dose response relationships for the activity of amantadine indicate that doses of 200 to 300 mg daily are optimal for most patients[43]. Schwab[36] noted some decreased effect after prolonged treatment.



VI

Amantadine has been successfully administered concurrently with other drugs, including levodopa whose efficacy it tends to predict[36].

Amantadine is generally well tolerated in most patients[44,45] and causes fewer side effects than levodopa or anticholinergics. Some reported side effects may be due to concurrent anticholinergic medication or to amantadine intensification of their effects, but this is not yet clearly established. Livedo reticularis has been reported[46].

Vernier et al.[47] concluded that amantadine was adequately tolerated on long term chronic administration to rats, dogs and monkeys at doses more than 13 to 33 times the usual human doses with no evidence of organ pathology. These toxicological studies showed some central nervous system stimulation, anorexia, rare emesis and some convulsions at high doses. Amantadine caused several effects at high doses, indicative of central nervous system stimulation or catecholamine interaction including: increased spontaneous motor activity, antagonism of tetrabenazine-induced sedation, a mild, transient vasodepressor effect, some cardiac arrhythmias, and some block of uptake of norepinephrine into labile stores (potentiation of norepinephrine vasopressor effect, block of phenethylamine vasopressor response, increase of myocardial contractile force, radioactive

norepinephrine distribution studies, and antagonism of tetrabenazine effects).

The possible modes of action of amantadine and other drugs in parkinsonism are summarized in Table 1. Amantadine does not act like the centrally-acting anticholinergics[48,49]. In three animal preparations amantadine failed to cause anticholinergic effects (guinea pig ileum, acetylcholine-induced depressor effects in dogs, oxotremorine tremor in mice).

Amantadine interacts with catecholamines in the central nervous system and in the periphery. This might be predicted from its primary amine structure. How can these interactions contribute to its effect in parkinsonism? Table 1 lists five mechanisms by which amantadine and other drugs could counteract the deficit of extrapyramidal inhibition which is responsible for symptoms of parkinsonism.

Recent evidence points to a local release of catecholamines as the major mode of action of amantadine in parkinsonism. This is the *most* sensitive effect of amantadine reported to date and occurs at the lowest dose. Although earlier clues pointed to this action, the results of Grelak et al.[48] clarified this point. They reported that a small transient pressor effect of amantadine alone, intravenously in dogs, was markedly intensified by dopamine-priming. This suggested that amantadine released dopamine and/or other catecholamines from neuronal stores to cause the peripheral pressor action. The effect was noted at very low amantadine doses. There was no evidence for block of dopamine uptake. Earlier Vernier[47] reported that transient increases in myocardial contractile force occurred following moderate intravenous doses of amantadine. These also suggested local catecholamine release, since they were abolished by reserpinization and restored by norepinephrine infusion.

Recently several laboratories have reported evidence for amantadine-induced release of radiolabelled catecholamine (mainly dopamine) from rat brain[50,51,52]. This seems to confirm in the central nervous system what was seen in the periphery. Although this may be the most likely major mode of action of amantadine in parkinsonism, the quantitative relations between this and other biochemical actions upon catecholamines remain to be worked out.

It is interesting to note that several authors have shown that levodopa may release amines in the brain, specifically serotonin, which may imply that it shares a somewhat similar action with amantadine[53,54].

Recent biochemical findings suggest that under some conditions amantadine may increase dopamine accumulation in brain. Thus Scatton[50] concluded that amantadine may stimulate dopamine synthesis and Stromberg[51] has reported compatible data. It is too early to assess the relevance of this finding to the clinical efficacy of amantadine.

Does amantadine block the reuptake of catecholamines and is this

effect related to its antiparkinson action? Several groups have studied the effect of amantadine upon catecholamine uptake since Vernier[47] reported data compatible with reuptake blockade. At doses 5-10 times the human therapeutic dose amantadine antagonized tetrabenazine-induced sedation in mice. It also potentiated norepinephrine-induced pressor response in dogs, blocked pehenethylamine-induced pressor response in dogs, and blocked the uptake of radiolabelled norepinephrine by mouse heart. In contrast Grelak[48] reported no clear indication of amantadine potentiating dopamine in their release experiments.

It is not yet clear whether amantadine blocks uptake of dopamine (or other catecholamines) in brain to prolong the useful presence of transmitter. While uptake block of dopamine and norepinephrine has been reported at high amantadine doses, it probably does not play a major role and the evidence of several groups is against it[50-55]. The quantitative and qualitative relations must be worked out before the role of reuptake block of catecholamines can be assessed. Snyder[57] suggested that catecholamine uptake blockade (mainly block of dopamine uptake by striatum) may contribute to the mode of action of anticholinergics and antihistamines in antiparkinsonian therapy.

Since amantadine generally lacks the prominent gastrointestinal symptoms (nausea and emesis) shared by apomorphine and levodopa it is not likely that it stimulates dopamine receptors directly.

Zetler[49] and Simon, Malatray and Boissier[56] have reported amantadine antagonism to phenothiazine-induced and other drug-induced catalepsy. They suggest that amantadine may be effective in drug-induced parkinsonism or other extrapyramidal disorders in man.

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TABLE 1 - MECHANISMS OF ACTION OF ANTIPARKINSONISM DRUGS.

ACTION	DRUGS	EVIDENCE
I. INCREASE EXTRAPYRAMIDAL INHIBITION		
(Restore Deficient Central Adrenergic Transmission - Mainly Dopamine)		
A. SUPPLY PRECURSOR	LEVODOPA	HORNYKIEWICZ[4]; BARBEAU[9]; COTZIAS[11,12]
B. RELEASE LOCAL TRANSMITTER	AMANTADINE	GRELAK[48]; VERNIER[47]; SCATTON[50]; BALDESSARINI[52]; STROMBERG[51]; OTHERS
C. INCREASE TRANSMITTER SYNTHESIS	AMANTADINE	SCATTON[50]; STROMBERG[51]
D. PROLONG TRANSMITTER AVAILABILITY	ANTIHISTAMINES	SNYDER[57]
BLOCK DOPAMINE UPTAKE	ANTICHOLINERGICS	SNYDER[57]
E. ACTS AS TRANSMITTER	APOMORPHINE	SCHWAB[22]; VERNIER[24]
II. DECREASE EXTRAPYRAMIDAL FACILITATION		
(Block Central Acetylcholine Muscarinic Transmission)	SCOPOLAMINE ATROPINE BENZTROPINE TRIHEXYPHENIDYL OTHERS	EXTENSIVE CLINICAL AND PHARMACOLOGICAL EVIDENCE - DUVOISIN[58]; VERNIER[59]

Section II - Pharmacodynamic Agents

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

Chapter 6. Antihypertensive Drugs

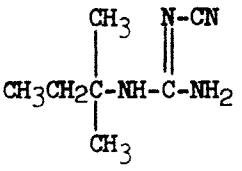
Fred M. Hershenson, G. D. Searle & Co., Chicago, Illinois

No new antihypertensive agents were marketed in the United States in 1970, although several compounds progressed through clinical trials and pose as potential products. Several clinical comparisons[1-3] of currently available antihypertensive agents appeared along with an excellent review[4] on the subject of antihypertensive drugs. In addition, the role of renin in hypertension was discussed in detail.[5]

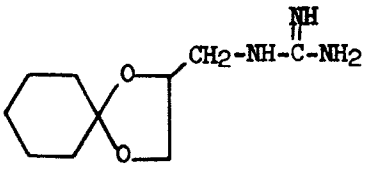
The spontaneously hypertensive rat (SHR) was reported as a useful model to screen for antihypertensive drugs. Methyldopa and MAO inhibitors (pargyline) are active in the SHR, but are inactive in normotensive animals.[6] The SHR is more responsive than renal-hypertensive rats to agents which depress sympathetic activity (reserpine, guanethidine).[7] Unlike DOCA-salt treated rats, which have decreased cardiac norepinephrine (NE) concentration and an increased cardiac NE turnover, the SHR has unchanged endogenous levels of NE in the heart, brainstem, and ileum, and a decreased rate of NE synthesis in the heart and brainstem.[8]

Compounds Under Clinical Evaluation - Guancydine (I) resembles hydralazine more than any other hypotensive agent in its hemodynamic effects. While the occurrence of fluid retention and tachycardia preclude its long-term use as a single agent, combination with reserpine and a diuretic has provided effective control of blood pressure with minimal side-effects.[9] Guancydine has also been used effectively in guanethidine-resistant hypertension.[10]

Guanadrel (II) exerts a guanethidine-like response with only one-third the potency on a weight basis. Side-effects include orthostatic hypotension and a significant reduction in renal function in the erect position. Unlike guanethidine, guanadrel reportedly does not cause diarrhea.[11]



I

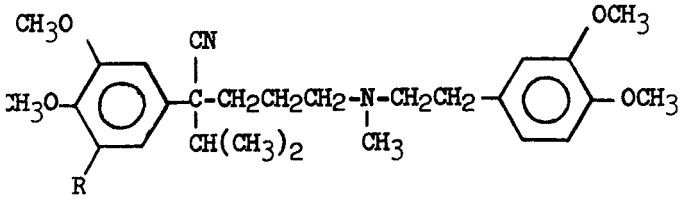


II

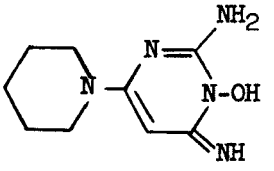
Verapamil (Isoptin®) (III) provides a rapid onset of activity at 5 mg, i.v., in renal-hypertensive patients with only a slight effect on

heart rate.[12] A single, oral dose of 1.8 mg/kg of compound IV lowered systolic and diastolic blood pressures 20 minutes after administration with peak activity occuring 2½ hours later.[13] Clinical evaluations of these compounds are continuing.

Although PDP (V) alone produces a reduction in blood pressure without orthostatic hypotension, the concurrent administration of a β-adrenergic blocker such as propranolol is required to control reflex increases in cardiac activity.[14] Hydrochlorothiazide has also been used with PDP to prevent sodium retention.

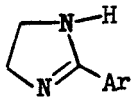


III (R=H)
IV (R=OCH_3)

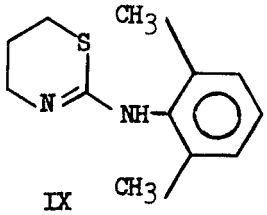


V

Clonidine (Catapres®) (VI) continues to represent an interesting new approach to the treatment of hypertension. Its most common side-effects, sedation and dry mouth, have been overcome by the combination of clonidine with chlorthalidone.[15] Unlike guanethidine and reserpine, clonidine does not cause orthostatic hypotension. This has been attributed to its ability to leave the vasoconstrictor reflex intact.[16] A series of clonidine analogs have been examined for hypotensive activity without the unwanted CNS effects. Two derivatives, St 600 (VII) and St 608 (VIII) have been selected for further study.[17]



VI (Ar=2,6-Cl_2-C_6H_3)
VII (Ar=2-CH_3 - 5-F-C_6H_3)
VIII (Ar=2-Cl - 3-CH_3-C_6H_3)

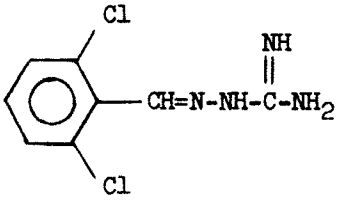


IX

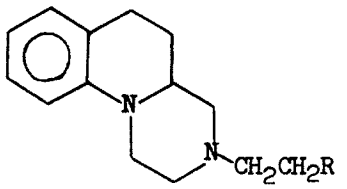
New Compounds (Central Activity) - Bayer-1470 (IX) produces a brief hypertension followed by a long-lasting decrease in blood pressure in anesthetized dogs at 0.25-1.0 mg/kg. Its mechanism of action, involving reduction of sympathetic tone by predominately central effects, is similar to that of clonidine.[18]

Wy-8678 (X), active at 0.5 mg/kg in unanesthetized rats and dogs, also displays the characteristic profile of activity involving inhibition of sympathetic tone at central sites.[19]

Incorporation of an o-tolylpiperazino moiety into a rigid molecular framework provided compound XI which displayed hypotensive activity at 1-5 mg/kg, i.v., in anesthetized cats. While XI has predominately a central site of action, compound XII acts peripherally.[20]



X

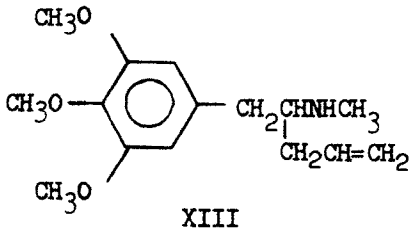


XI (R=C_6H_5)

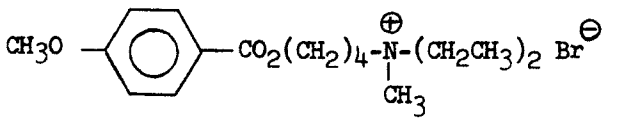
XII(R=4-F-C_6H_4-C(O)CH_2)

In a series of 38 aralkylbutenylamines, compound XIII provided the best hypotensive activity; 50 mg/kg, orally, in renal-hypertensive dogs.[21]

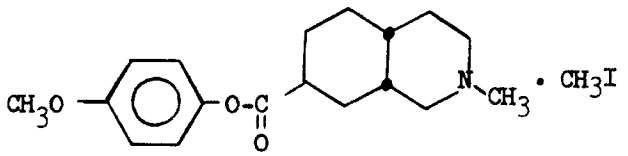
Compound XIV, active at 1 mg/kg, i.v. in rabbits, is reported to display a hypotensive activity similar to reserpine.[22] The decahydroisoquinoline derivative, XV, was also reported to have reserpine-like activity.[23]



XIII



XIV

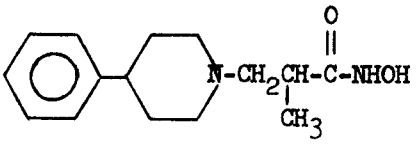


XV

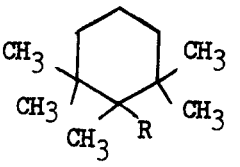
The antihypertensive activity of both dl- and d-propranolol given intraventricularly to anesthetized cats suggests a central component to the hypotensive response that is independent of the β-adrenergic blocking activity.[24]

New Compounds (Ganglion Blockers) - K76 (XVI) produced hypotensive activity in anesthetized cats via blockade of sympathetic ganglia, and is as effective as pentolinium bitartrate.[25] Examination of a series of related N-alkyl and N,N,-dialkyl-aminopropionhydroxamic acids failed to provide a compound with more potent activity.[26]

While the unbridged analogs of mecamylamine, XVII and XVIII had only one-tenth the ganglionic blocking activity of mecamylamine,[27] a series of tertiary hexylamines were found to be more potent in anesthetized cats. In addition, compound XIX had twice the hypotensive activity of mecamylamine with only 20% the toxicity.[28]



XVI



XVII (R=NH_2)

XVIII (R=$NHCH_3$)

CH_3-$C(CH_3)_2$-$C(CH_3)_2$-$N(CH_3)_2$

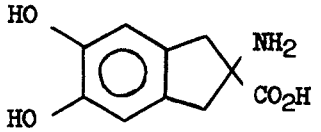
XIX

New Compounds (Catecholamine Biosynthesis) - Several reports appeared involving agents which would disrupt the biosynthesis of norephinephrine, specifically at the first step by the inhibition of tyrosine hydroxylase. A series of halogenated phenylalanines were found to inhibit both tyrosine and phenylalanine hydroxylase *in vitro*. α-Methylation enhanced the tyrosine hydroxylase inhibition activity of the 3-halophenylalanines without affecting phenylalanine hydroxylase inhibition.[29]

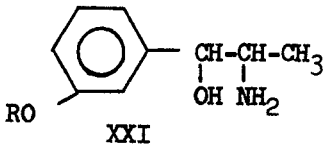
A microbial product, Oudenone, obtained from a strain of microorganisms related to *Oudemansiella radicata*, was reported to possess tyrosine hydroxylase inhibition activity. Oudenone also had hypotensive activity in spontaneously hypertensive rats at not less than 3.13 mg/kg, i.p.[30]

Deoxyfrenolicin, an analog of the antibiotic frenolicin, is also a potent tyrosine hydroxylase inhibitor *in vitro* and *in vivo*, although no mention of its possible antihypertensive activity was made.[31]

α-Methyl dopa does not seem to exert its antihypertensive effects by producing a weakly active false sympathetic neurotransmitter.[32] Its activity is not blocked by adenoreceptor or ganglionic blocking agents or by dopamine β-oxidase inhibitors. The cyclic analog of α-methyl dopa, compound XX, did not inhibit dopa decarboxylase *in vitro*, and was inactive in DOCA and renal-occluded, hypertensive rats.[33]



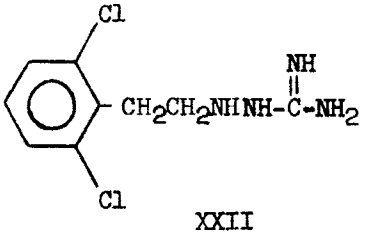
XX



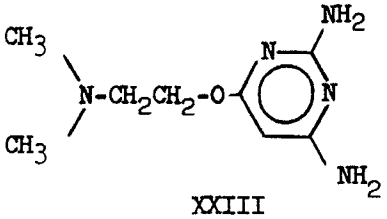
XXI

Examination of the optical isomers of metaraminol (XXI, R = H) showed that only the (1R,2S) or (-) erythro isomer produced catecholamine depletion and antihypertensive activity.[34] A series of metaraminol ethers, found to cause norepinephrine depletion, are dealkylated *in vivo* to metaraminol.[35]

New Compounds (Miscellaneous) - The tautomerism and conformations of the benzothiadiazine antihypertensive agents were predicted by means of Extended Huckel Molecular Orbital calculations.[36] Speculations on the nature of the electronic molecular mechanism of action and the structure of the receptor were also made.[37]



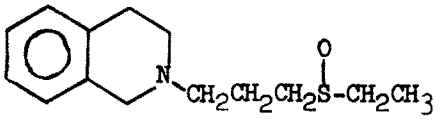
XXII



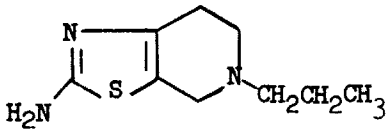
XXIII

Several aralkylaminoguanidines were found to exert adrenergic neuron blocking activity without causing depletion of cardiac norepinephrine stores. Compound XXII, which shows hypotensive activity at 50-225 mg in man, has been selected for clinical evaluation.[38]

SKF-11197 (XXIII) produces its antihypertensive effects by reducing peripheral resistance *via* direct vasodilatation. At 5-10 mg/kg, i.v., SKF-11197 produced hypotension and tachycardia in anesthetized dogs and cats, and in unanesthetized, normotensive and renal-hypertensive dogs.[39]



XXIV



XXV

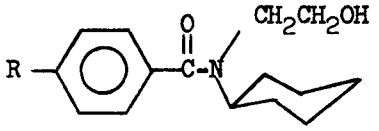
NC-7197 (XXIV) was found to be active at 0.3-1.0 mg/kg in anesthetized dogs. The hypotensive effects of NC-7197 were due to the compound's competitive α-adrenergic blocking activity.[40]

The antihypertensive action of BHT-324 (XXV) parallels the accumulation of norepinephrine in the heart, and is attributed to a decreased activity of the sympathetic nervous system.

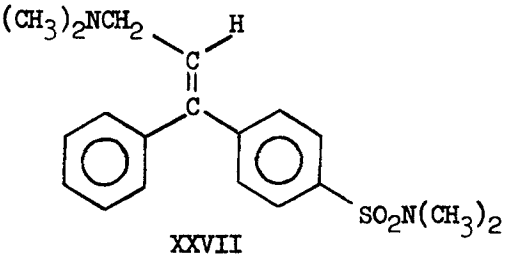
Derivatives of β-hydroxyethylcyclohexylamine, (XXVI, R = Cl, OCH_3, NO_2) produce CNS depression and hypotension in normotensive rats at 4 mg/kg.[42,43] These compounds display a rapid onset and short duration of activity.

Compound XXVII was reported active at 1 mg/kg, i.v., in anesthetized

and unanesthetized dogs and active orally in renal and neurogenic hypertensive dogs.[44] The hypotensive activity appears to be related to histamine-releasing properties of the compound.



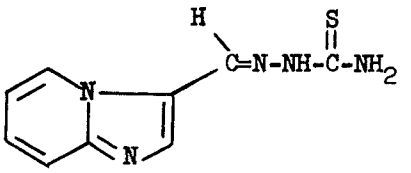
XXVI



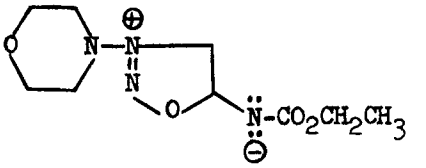
XXVII

The thiosemicarbazone of 3-formylimidazo [1,2-a] pyridine, XXVIII, produced antihypertensive activity at 2.5-10 mg/kg, i.v., in anesthetized dogs, and at 25-100 mg/kg, orally, in unanesthetized dogs. Pulmonary edema was also observed with XXVIII, probably resulting from a change in pulmonary hemodynamics.[45]

SIN-10 (XXIX), at 1-5 mg/kg, orally or i.v., in anesthetized dogs, produced a gradual and prolonged fall in blood pressure, along with decreased cardiac output and increased respiratory rate.[46]



XXVIII



XXIX

Viopudial, isolated from Viburnum opulus, was reported to have hypotensive and smooth muscle antispasmotic properties.[47] This sesquiterpene dialdehyde decreased arterial blood pressure at 2 mg/kg in dogs.

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Chapter 7. Platelet Aggregation Inhibitors

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Introduction - The therapeutic approach to thromboembolic disease which is based on the inhibition of platelet aggregation was previously reviewed as part of an annual report on the broader topic of agents affecting thrombosis.[1] The reader is referred to this previous work for information on the search for agents that act by fibrinolytic or anticoagulant mechanisms.

Consideration of thrombus formation as a probable common manifestation of many cardiovascular diseases, such as myocardial infarction and stroke, leads one to the conclusion that thromboembolism may be the most prevalent disease entity in our middle-aged population. An approach to the treatment of these cardiovascular diseases that is based on a common mechanism would seem to be the most productive course to pursue, and it is for this reason that the management of thrombosis has emerged as an important therapeutic objective in recent years. The use of fibrinolytic agents for the dissolution of thrombi and the prophylactic use of anticoagulants for their prevention has been known for some time, but success with these agents has been significant only in the management of venous thrombosis in which coagulation factors, not platelets, play an important role. Arterial thrombi, which are essentially composed of aggregated platelets and are not blood clots, are not affected by anticoagulants. Indeed, these drugs have not been proved effective in the prevention of disease states such as myocardial infarction which are believed to be due to arterial thrombosis. In recent years, the approach to the management of arterial thromboembolism based on knowledge of the factors affecting platelet function has gained prominence. The rationale behind this approach is based on the hypothesis that platelet aggregation is an early event in the formation of a thrombus and that inhibition of this aggregation in a specific manner without substantially altering hemostasis would constitute an effective method for the prevention of thrombosis. This point has been elaborated extensively in recent articles by Mustard and Packham,[2,3] who have also recently compiled a comprehensive review on blood platelets.[4] The present report will attempt to present the highlights of research in this area during 1970.

By way of introduction, it may be useful to reiterate briefly some of the salient features of the platelet role in hemostasis and thrombosis which are developed in detail in the reviews mentioned above. The principal mechanisms involved are the platelet release reaction, ADP-induced platelet aggregation, and blood coagulation. Platelets are induced to aggregate by various substances in response to stimuli or injury. Some of the best known of these substances are ADP, collagen, thrombin, and epinephrine. Under aggregating conditions platelets undergo a reaction which results in the release of blood coagulation factors, serotonin and ADP among other substances. Released ADP induces

further aggregation.

Prostaglandins and Cyclic AMP - The activities of prostaglandins as inhibitors of platelet aggregation have been reviewed.[1] The most conspicuously active member of this group of compounds is PGE_1 which is at least 100 times more potent as an inhibitor of human platelet aggregation than the next most active member, PGE_2. Neither PGE_1 nor PGE_2 has an effect on rat blood coagulation *in vitro*[5]. Prostaglandins have been found to be products of the platelet release reaction. At least two prostaglandins, PGE_2 and $PGF_{2\alpha}$, were released from human platelets stimulated with thrombin, but it is interesting to note that no detectable amount of PGE_1 was reported.[6]

The action of PGE_1 on platelets is thought to be mediated by intracellular cyclic AMP levels. The evidence that PGE_1 stimulates platelet adenyl cyclase, and thereby increases cyclic AMP levels has been accumulated in several laboratories.[7-11] Adenyl cyclase activity of platelet homogenates was found to be increased by PGE_1 and NaF, and decreased by collagen, serotonin and thrombin. The enzyme activity was not affected by ADP, cyclic AMP or dibutyryl cyclic AMP. Both initial aggregation and the release reaction of intact platelets induced by collagen, thrombin, ADP, and epinephrine were inhibited by dibutyryl cyclic AMP. Cyclic AMP was also active but less effective. This was attributed to the possible superior membrane penetrating ability of dibutyryl cyclic AMP.[7] Both PGE_1-stimulated and basal cyclic AMP synthesis rates were inhibited by epinephrine in intact platelets and platelet membrane fractions. This effect was blocked by phentolamine but not by propranolol consistent with the involvement of α-adrenergic receptors. The possibility that the epinephrine effect was due to ATP depletion was unlikely because inhibition of cyclic AMP synthesis was independent of ATP concentration. Also, epinephrine had no effect on the activity of isolated phosphodiesterase.[8] It appears that compounds that cause a rise in intracellular cyclic AMP levels generally inhibit the platelet release reaction. This is exemplified by adenyl cyclase stimulators such as PGE_1 on the one hand and by phosphodiesterase inhibitors such as theophylline on the other. Both compounds inhibit platelet aggregation and the release reaction.[9] Phosphorylase *a* activity in platelets has been assayed before and after thrombin or epinephrine treatment, and this enzyme does not appear to be modulated by a direct effect of intracellular cyclic AMP levels as it is in other cells.[10] Collagen-induced aggregation is inhibited in a dose-dependent manner by PGE_1, theophylline, and aspirin. Recent studies of adenine nucleotide levels in platelets utilizing ^{14}C-labeled adenine have revealed that inhibition of the release of ADP and ATP, as well as ATP breakdown, reflects the same dose-dependent relationship. PGE_1 stimulated the synthesis of radioactive cyclic AMP. These studies revealed the somewhat

unexpected result that theophylline did not affect cyclic AMP levels by itself; however, theophylline and PGE_1 exhibited a synergistic effect on cyclic AMP synthesis and inhibition of aggregation. The importance of cyclic AMP levels as the modulator of aggregation was diminished by the finding that both compounds caused significant inhibition of platelet aggregation at low dose levels which caused no appreciable change in cyclic AMP levels.[11] On the other hand, further evidence that aggregation is inhibited by raised cyclic AMP levels is provided by reports of the coincidence of aggregation and phosphodiesterase inhibitory activity in the same compounds. For example, many potent phosphodiesterase inhibitors such as theophylline, 2-chloroadenosine, promethazine and chlorpromazine are inhibitors of platelet aggregation.[12] Papaverine and its derivatives have been shown to possess both types of inhibitory activity.[13] Although further investigation is required to establish the point firmly, the majority of experimental evidence is consistant with the hypothesis that platelet aggregation is regulated by intracellular cyclic AMP levels and that PGE_1 inhibits aggregation by stimulating platelet adenyl cyclase.

Aspirin and Other Nonsteroidal Anti-Inflammatory Agents - Many nonsteroidal anti-inflammatory (NSAI) agents have been found to be inhibitors of platelet aggregation. Studies designed to compare the relative potency of these compounds on platelets have generally shown that aspirin and indomethacin are the most potent inhibitors in this class, and compounds such as mefenamic acid and phenylbutazone are much less potent.[14,15] The NSAI agents have a common mode of action on platelets. That is, they inhibit aggregation by preventing the platelet release reaction. Thus, aggregation induced by collagen, antigen-antibody complexes, epinephrine and low concentrations of thrombin is inhibited, but ADP induced aggregation is not inhibited. Also, consistant with this mode of action the "second wave" of ADP induced aggregation due to released endogenous ADP is abolished.[4] The NSAI agents are generally one to two orders of magnitude less active than very potent inhibitors of ADP-induced platelet aggregation such as adenosine.

Although sulfinpyrazone is used clinically only as a uricosuric agent, its effect on platelet function is similar to that of the NSAI agents and it has been investigated in this regard.[16] Along with aspirin and dipyridamole, sulfinpyrazone is one of the only three compounds which have undergone significant clinical evaluation as antithrombogenic agents in man. Platelet survival time is shortened in patients with artificial heart valves, and this is associated with platelet aggregation and eventual thromboembolism. A daily oral dose of 800 mg of sulfinpyrazone has been found to correct platelet abnormalities in these patients.[17]

There has been considerable interest recently in studying aspirin as an antithrombogenic agent because of the duration of action of this compound. The *in vivo* inhibitory effect in man of a single 1-2 g dose has been observed to last for 2-7 days.[18] Aspirin has been observed to

inhibit the release of pharmacologically active substances from a variety of tissues.[19] Inhibition of the platelet release reaction may be one local manifestation of this general property. The effect of aspirin and other NSAI agents on the release of ^{14}C-serotonin from human platelets induced by connective tissue particles has been studied.[14] These data indicated that platelets may exhibit two mechanisms for serotonin release because under the condition of maximal inhibition by aspirin further release of serotonin could be elicited by raising connective tissue particle concentration. Based solely on the relative inhibition of ^{14}C-serotonin release, indomethacin was found to be more potent than aspirin. The release of platelet factor 4, heparin neutralizing activity (HNA), in response to exogenous ADP is inhibited by aspirin.[20] Both the formation of initially formed platelet bound HNA and the release of soluble HNA associated with the platelet release reaction are blocked by aspirin. Aspirin also inhibited the release of platelet factor 3 from human platelets induced by ADP. Platelet factor 3 release appears to depend on the degree of aggregation and does not parallel the release of serotonin or endogenous ADP.[21]

The mechanism of inhibition of the platelet release reaction by aspirin is believed to involve acetylation of the platelet membrane, and this alteration of the membrane irreversibly alters platelet function. This would explain the long duration of action observed *in vivo* with aspirin. The experimental facts consistent with membrane acetylation have been summarized in a recent report.[22] Briefly, these are as follows: (1) acetic anhydride inhibited platelet aggregation in an aspirin-like manner, (2) salicylic acid is relatively inactive as an inhibitor, and (3) radioactivity was incorporated into platelets from aspirin labeled with ^{14}C in the acetyl group, but no incorporation of label was observed with aspirin labeled in the salicylate portion of the molecule. The possible incorporation of labeled acetate from the acetate pool resulting from a prior rapid hydrolysis of aspirin could not be excluded by the available experimental data. It has been shown that aspirin and other NSAI agents do not block glucose uptake or $^{14}CO_2$ formation by unstimulated platelets.[16] Furthermore, these compounds prevented the thrombin- and collagen-induced $^{14}CO_2$ production associated with the energy expended in aggregation, but did not affect increased $^{14}CO_2$ production induced by exogenous ADP. These observations are consistent with the hypothesis that the action of NSAI compounds is not due to an effect on platelet metabolism.

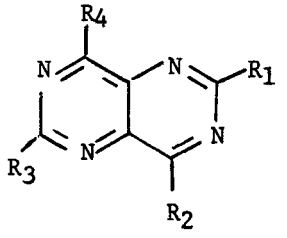
A number of reports on *in vivo* studies of the antithrombogenic properties of aspirin have appeared recently. In this regard, it is important to realize that the validity of animal models of thrombosis remains open to question. Platelets from aspirin treated rabbits were found to be morphologically normal, but did not aggregate in the presence of collagen *in vitro*.[23] Thrombus formation induced in rats by *S. typhosa* endotoxin was inhibited by aspirin previously administered by stomach tube.[24] Platelets from these animals exhibited a reduced tendency to aggregate *in vitro* and the prevention of thrombus formation could be

associated with a decrease in platelet aggregation. Early investigations in man were carried out on patients receiving a daily oral dose of 1-2 g of aspirin. Under this treatment patients with abnormally enhanced platelet aggregation exhibited an inhibition of platelet aggregation, and a rapid clinical improvement was seen in patients with thromboembolic diseases.[18] Because of undesirable side effects associated with large oral doses of aspirin, which are exemplified in particular by gastric complaints and hemorrhages, the dose requirements have been examined. Recent investigations in human subjects have indicated that a 300 mg per day oral dose of aspirin has a significant effect on platelet function; no greater effect could be obtained from larger doses.[25] Significant inhibition of collagen-induced aggregation and a reduced tendency for the platelets to adhere to glass were observed. Also, no accumulation of plasma salicylates and no significant side effects were found in these subjects. The effects of aspirin on platelet function have been demonstrated to persist during long term administration.[21] Comparison of platelets from patients with certain bleeding disorders with those from aspirin treated normal subjects shows similarities.[26] Impaired collagen-induced aggregation associated with a decreased release of platelet ADP was exhibited in both cases, but the aspirin effect was less pronounced. Aspirin potentiated the effect of dipyridamole in the prevention of platelet interaction with artificial heart valves, but it had little effect when administered alone.[27] Aspirin has been observed to prolong platelet survival in man.[3]

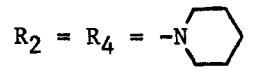
In summary, the platelet inhibitory properties of aspirin appear to be due to its ability to acetylate proteins, and thereby cause an irreversible change in the platelet membrane which inhibits aggregation. Therefore, the duration of inhibitory action roughly parallels platelet survival time and is much longer than expected on the basis of the short serum half-life of this drug. Relatively low potency has been observed *in vitro*, and the question of effective antithrombotic dosage *in vivo* remains to be settled in clinical investigations. Large oral doses may be prohibitive over long periods because of undesirable gastric and other side effects.

Other Inhibitors - Pyrimidopyrimidines are well known inhibitors of platelet aggregation, and dipyridamole (Persantine) is the oldest and most thoroughly studied of these compounds.[1-4] Dipyridamole inhibits ADP-induced aggregation and serotonin release induced by collagen. The effect on the release reaction appears to be different from that of aspirin because dipyridamole caused a spontaneous loss of ^{14}C-serotonin from platelets, whereas aspirin did not.[14] It has recently been shown that inhibition of ADP-, collagen- or thrombin-induced aggregation of human, pig or rabbit platelets with dipyridamole and related analogs is accompanied by inhibition of glucose uptake and depressed $^{14}CO_2$ production from glucose-6-^{14}C. This suggests that these compounds, as distinct from aspirin and other NSAI agents, exert their action by an effect on platelet metabolism.[16] Since adenosine is a potent inhibitor of platelet aggregation and dipyridamole blocks the uptake of adenosine by platelets, it has also been suggested that dipyridamole

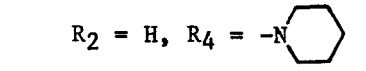
may act by increasing platelet membrane adenosine concentration.[28] Recent clinical studies in patients with artificial heart valves have shown that platelet survival time is decreased by an amount directly related to the surface area of the valve. Dipyridamole prevented this effect, but no impairment of platelet function could be demonstrated in vitro with blood samples from the treated patients.[27]

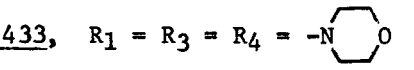


Dipyridamole, $R_1 = R_3 = -N(CH_2CH_2OH)_2$

$R_2 = R_4 =$ 

RA 233, $R_1 = R_3 = -N(CH_2CH_2OH)_2$

$R_2 = H$, $R_4 =$ 

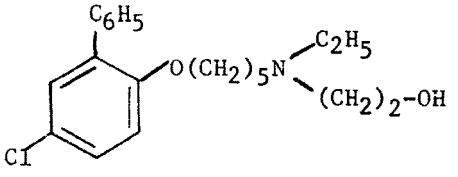
RA 433, $R_1 = R_3 = R_4 =$ 

$R_2 = H$

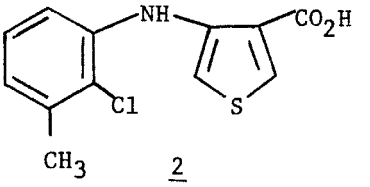
Dipyridamole has hypotensive activity and has been studied extensively as a coronary vasodilator.[29] In this respect it is similar to other vasoactive agents such as adenosine and PGE_1 which also inhibit both ADP-induced aggregation and the release reaction of platelets. Efforts to obtain dipyridamole derivatives with enhanced effects on platelets relative to vasodilator activity have produced the two analogs RA 433 and RA 233. Recent in vitro studies have shown that both are more potent than dipyridamole as inhibitors of ADP-induced aggregation of human platelets. Both compounds were highly active in a variety of platelet function tests and the in vitro evidence indicated that RA 233 was the more potent of the two.[30] In contrast to these results, in vivo studies in rats have indicated that RA 433 is more effective than RA 233 in the inhibition of thrombogenesis. Furthermore, RA 433 produced both an antithrombogenic effect and a hypotensive effect at lower doses, and exhibited a more favorable ratio of antithrombogenic to hypotensive properties than RA 233. In the rat, RA 433, RA 233 and dipyridamole all exhibited a significant hypotensive effect at dose levels required for an inhibition of thrombogenesis.[28]

Three new structural types of platelet aggregation inhibitors which have appeared recently are exemplified by compounds 1, 2 and 3. Compound 1 was the most active of a series of analogs which inhibited ADP-induced

aggregation of rabbit platelets in vitro. None of these analogs were as active as adenosine, but the inhibitory activity was comparable to that



1



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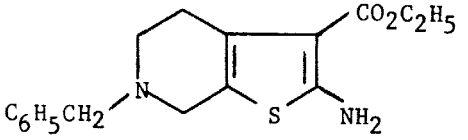

3

of known tricyclic antidepressants and antihistamines.[31] Investigation of a variety of phenyl derivatives of 4-aminothiophene-3-carboxylic acid, which may be regarded as isosteres of mefenamic acid, revealed that compound 2 was the most potent as an inhibitor of collagen-induced aggregation of human platelets in vitro. A number of these analogs also exhibited fibrinolytic activity at concentrations at least one order of magnitude higher than those required for inhibition of platelet aggregation. Structural changes which increased fibrinolytic activity reduced activity on platelets.[32] In addition to analgesic, antipyretic and anti-inflammatory activity, compound 3 was reported to be a potent inhibitor of ADP-induced platelet aggregation.[33]

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Chapter 8. Agents Affecting Gastrointestinal Functions

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This review covers the two-year period, 1969-1970. As Hess noted in the previous review in this series,[1] the bulk of drug research is still directed primarily toward agents for the treatment of peptic ulcer disease. It seems to us, however, that the approaches being used are now oriented more toward the investigation of basic physiologic controlling mechanisms (hormones, prostaglandins, CAMP) which could lead to completely new types of therapeutic agents.

Gastrointestinal Hormones

The great interest in these compounds and the large amount of ongoing research on them is reflected by the number of reviews which were published during the last two years. Gastrin--its myriad effects, measurement, and metabolism--has been the subject of several reviews;[2-6] the literature on secretin,[7] cholecystokinin (CCK)[7,8] and related caerulein,[9] and glucagon[10] has also been summarized. Gregory[11] briefly reviewed gastric secretory hormones and chalones, and Brooks[12] reported on a gastrointestinal hormone research symposium sponsored by the Nobel Foundation (proceedings to be published by Wiley-Interscience).

Gastrin - Early structure-activity studies on gastrin showed that, of the biologically active tetrapeptide sequence, the aspartic acid position was most sensitive, substitutions at that position being devoid of activity. Now Morley[13] has shown that the β-carboxy group of Asp can be substituted by a tetrazolyl group which has similar spacial and electronic characteristics and that the resultant compound retains the biological activity of the parent compound. Substitution of the Met group by norleucyl or 4-dehydroleucyl produced active tetrapeptides; these compounds were made as intermediates in the preparation of the tetrapeptide labeled with tritium in the Asp group.[14]

Our knowledge of the distribution and metabolism of gastrin has been expanded by a number of studies. By bioassay, the half-life of both pentagastrin and synthetic human gastrin I was found to be quite brief, 1.5 and 2.65 minutes, respectively; this was attributed to diffusion into extracellular water as well as to destruction and/or elimination.[15] Pentagastrin is inactivated by serum, but not rapidly enough to account for this brief half-life.[16] Pentagastrin has also been reported to be inactivated by rat liver homogenate[16] and by passage through the liver in man,[17] but little inactivation of endogenous gastrin was attributed to this mechanism.[18] Kidney is apparently capable of destroying gastrin,[19] but lung is not.[20] Although endogenous gastrin is found in thoracic duct lymph after release, this does not seem to be its major route to the

peripheral circulation.[18]

A number of attempts have been made to compare the potency of different gastrins and related compounds, but because of the different techniques used the studies cannot be compared among themselves. Pure porcine gastrins I and II gave almost identical dose response curves, as did caerulein and CCK; the gastrins were much more potent. Comparative potencies for the two pairs of compounds could not be determined, however, because the curves were not parallel.[21] In contrast to findings *in vivo*,[22] pentagastrin was found more potent than the whole molecule *in vitro*.[23] An important observation has been that gastrin loses some of its activity during the process of iodination used to label the compound for radioimmunoassay and distribution studies.[24]

Reviews on the development of radioimmunoassay techniques for gastrin point up their degree of refinement; one can now measure very low levels of gastrin (down to 1 pg) and differentiate species-specific gastrins.[5,6] The Yalow and Berson technique is the simplest and least expensive procedure, using crude porcine gastrin without conjugation to a carrier protein. With such methods, it has been demonstrated that in human plasma gastrin exists primarily as a complex (approximate mol. wt. 7000) of the heptadecapeptide and a more basic peptide.[4] Although there is a fairly wide range of gastrin concentrations which have been found in both normal subjects and peptic ulcer patients by the various methods used,[5,6,25,26] there is general agreement that gastrin blood levels are markedly elevated in patients with pernicious anemia or with Zollinger-Ellison syndrome. Immunoassay may become useful in diagnosis of the latter. Another potential clinical use for gastrin antibodies was suggested by the demonstration that such antibodies could inhibit the activity of endogenous gastrin.[27]

Studies with a series of aliphatic alcohols have shown that their effectiveness as local stimulants of gastrin release is primarily influenced by molecular configuration (length and shape of the carbon chain), ethyl and n-propyl alcohols being the most active.[28,29] Further studies with ethyl alcohol showed that activity was correlated with concentration and pH, falling off rapidly below pH 3. That pH influences the effectiveness of a stimulant of gastrin release is well known; recent data suggests that this may be attributable to the effect of pH on the ionization of the stimulant molecule as well as on the charge of the mucosal surface.[30]

Cholecystokinin (CCK) and Caerulein - The C-terminal octapeptide sequences of CCK and caerulein differ only in the presence of methionine or threonine in position 6. Since these compounds have been synthesized, a considerable number of structure-activity studies have been conducted by investigators at Squibb and at Farmitalia.[8,9]

	8	7		6	5	4	3	2	1
Cholecystokinin	--Asp--	Tyr(SO_3H)--		Met--	Gly--	Try--	Met--	Asp--	Phe--NH_2
Caerulein			--Thr--						

Both the position and sulfation of the Tyr residue are critical to cholecystokinetic activities of both molecules;[31,32] however, the sulfated tyrosine residue must be part of a peptide chain of certain length for optimal activity.[8] Contrary to experience with other peptide hormones, the C-terminal octapeptide of CCK is more potent than the total molecule on a molar basis.[8] Erspamer[9] has reported preliminarily that dissociation of some of the biological activities of caerulein have been accomplished by changing its structure. New in vivo and in vitro techniques for biological assay of CCK have been designed.[33,34]

The possibility of producing analogues of CCK which could be used clinically to block gastrin induced acid secretion but have little intrinsic secretory activity of their own has been preliminarily explored.[35] Such compounds could conceivably be administered intranasally[36] thus having an advantage over secretin which must be given by injection.

Secretin - The scope of physiological activities of secretin has been broadened by recent findings; it now seems fairly certain that these activities include stimulation of pepsin secretion[37] and of Brunner's gland secretion.[38] Since the availability of natural secretin of great purity and synthetic secretin, investigators have been able to demonstrate that, in contrast to older preparations, pure secretin is effective by the subcutaneous route[39] and up to ten times more potent.[40]

Glucagon - It has been known for some time that glucagon is capable of suppressing gastric secretion in man and animals;[10] it has even been referred to as a "physiologic antigastrin".[41] The mechanism of secretory inhibition by glucagon is currently undergoing some scrutiny in several laboratories; the inhibition does not appear to be mediated by hyperglycemia, nor by decreased serum calcium levels.[41] Although glucagon loses some of its activities after passage through the liver, its effect on gastric secretion remains even after intraportal administration.[42]

Gastrointestinal Chalones

Gastrone - At present the most purified material which exhibits gastrone activity is the glycoprotein of Glass and his coworkers. In recent investigations to determine its mechanism of action, they have found that in dogs the decreased gastric secretion induced by this material is associated with a decrease in mucosal blood flow. Both effects appear an hour after gastrone administration.[43] The antisecretory effect is also associated with pyrexia and leucopenia followed by leucocytosis, effects resembling those of bacterial endotoxins.[44]

Enterogastrone - Starting with a partially purified preparation of CCK-PZ, Brown and others[45] separated fractions which strongly inhibited acid and pepsin secretion, and gastric motor activity, but had no cholecystokinin or secretin activities. Amino acid analysis of the most potent antisecretory fraction revealed high concentrations of aspartic and glutamic acids, as well as lysine; proline, found in CCK-PZ, was absent.[46] The polypeptide was cleaved at its single methionine residue with cyanogen

bromide; subsequent testing of the C-terminal fragment showed it to have about one-half the activity of the whole molecule when tested against pentagastrin stimulated secretion in dogs.[47]

Another material prepared from hog intestine inhibited gastric secretion and motility but lacked the activities of other gastrointestinal hormones and was not pyrogenic.[48] This substance was effective against secretion submaximally stimulated by histamine in dogs, while purified preparations of CCK and secretin were not.[49]

Urogastrone - Purification and testing of urogastrone have been taken up by chemists and pharmacologists at Imperial Chemical Industries, Ltd. So far, their data indicate that urogastrone is a relatively small acidic polypeptide (M.W.= 4000) composed of a single chain with internal disulfide bonds; both ends of the molecule appear to be blocked.[11] Urogastrone inhibits gastric secretion stimulated by histamine and both exogenous and endogenous gastrin; it does not affect gastric motility, pancreatic exocrine secretion, or salivary secretion.[50]

Another urogastrone material, prepared from human pregnancy urine, has been studied for a number of years by a group at the University of Milan. The most active fraction appears to be a glycoprotein with little or no gonadotropic activity; it reduces gastric secretory volume and acid concentration, but has no effect on pepsin concentration in rats.[51]

Prostaglandins in Gastrointestinal Function

A number of studies on the occurrence, release, and metabolism of prostaglandins and their role in gastric secretion and gastrointestinal motility have been completed or are in progress.[52] The implication of CAMP in their mode of action remains of interest, and this topic has been the subject of recent reviews.[53-55]

Gastric Secretion - PGE_2, which is present in the gastric mucosa of man, reduced pentagastrin induced secretion in anesthetized rats when administered in hypotensive doses.[52] A synthetic PGE racemate inhibited basal and pentagastrin induced secretion in rats.[56] Another PGE_1 analogue (AY 20,524) exhibited one-tenth the activity of PGE_1, while PGF analogues were about one-eighth as active as AY 20,524.[57] For the first time PGE_1 was shown to suppress basal acid secretion in man.[58] PGA_1 also suppressed basal volume and acidity in man, although it appeared that the inhibition was not dose related, and not as effective in the hypersecreting individual.[59]

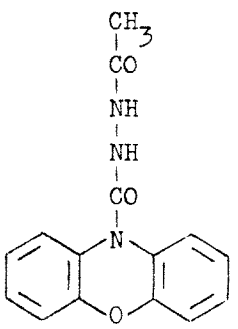
Controversy still prevails on the cause/effect relationship between antisecretory effects and blood flow changes. Some evidence suggests that PGE_1 has a direct effect on the secretory mechanism. PGE_1 inhibited pentagastrin or histamine stimulated secretion in dogs without depressing the mucosal blood flow to secretion ratio. In contrast, doses of norepinephrine which reduced secretion also reduced the blood flow/secretion ratio.[60] PGE_1 was also reported to inhibit histamine or pentagastrin

stimulated secretion in the frog gastric mucosal preparation.[52]

The possible interrelationships between prostaglandins and CAMP on acid secretion were further investigated. Harris and others demonstrated, according to several criteria, that methyl xanthines in the frog mediate secretion by accumulation of CAMP.[61] Although PGE_1 was capable of inhibiting gastrin and histamine induced secretion in the frog mucosa, it did not affect CAMP mediated secretion. However, it was also found that perfusion of PGE_1 over the mucosa of the rat stomach did inhibit gastric secretion produced by addition of CAMP.[52] Complicating the picture are data which indicate that both histamine and prostaglandin increase adenyl cyclase activity; gastrin, however, is without effect on this enzyme.[62]

Gastrointestinal Motility - Longitudinal muscle of small and large intestine contract in the presence of prostaglandins of the E and F types.[52] On the other hand, the F types generally cause contraction of circular muscle, whereas the E compounds produce relaxation.[63,64] The effect of prostaglandins on circular muscle appears to be direct, but the effect on longitudinal cells probably also involves non-anticholinergic excitatory nerves.[64] In man, PGE_1 produces bile reflux into the stomach, suggestive of an effect on antral motility as was observed in the dog.[52] In another study in man, PGE_1 increased propulsion and transit time, and produced colic and nausea.[65]

A better understanding of the function of prostaglandins in gastrointestinal motility could come from the availability of competitive antagonists. There is an indication that SC 19220 [1-acetyl-2-(8-chloro-10,11-dihydrodibenz [b,f][1,4] oxazepine-10-carbonyl) hydrazine] competitively inhibits the effects of PGE_2 induced contractions of guinea pig ileum.[66] A series of synthetic prostaglandin analogues has also been reported to compete for the same receptor site as prostaglandins.[67]



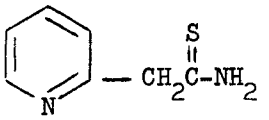
SC 19220

Agents with Antisecretory or Antiulcer Activity

Antisecretory Thioacetamides - SC-15396 [(2-phenyl-2-(2-pyridyl)- thioacetamide] was the subject of many publications over the time period of the previous review[1] but subsequently fewer. Recent findings reconfirm the earlier observations that this compound is an inhibitor of gastric secretion, more powerful against gastrin than against vagal or histamine stimulated secretion; it produces dose related suppression against all three challenges in the dog,[68] and dose related inhibition of

pentagastrin induced secretion in the rat.[69]

Another thioacetamide, 2-pyridyl-thioacetamide, was found to be a potent inhibitor of pentagastrin induced secretion in rats and dogs.[70] The compound also inhibits basal secretion in rats, and is very active in a variety of experimental ulcer techniques. Although the compound blocked the effect of pentagastrin on smooth muscle, no anticholinergic or antihistaminic activity was found. Up to the present, no structure-activity studies have appeared in the literature, nor is there evidence of activity of this type of compound in man.



2-pyridyl-thioacetamide

Depepsen - Sulfated amylopectin (SN-263, a sodium salt of sulfated potato amylopectin) is a potent inhibitor of pepsin proteolysis *in vitro*, and protects against certain experimentally induced ulcers in animals. Frequent, large doses in man enhanced healing rate of gastric ulcers as evidenced by measurement of ulcer size by serial radiography.[71] In a double blind study the effect of SN-263 alone and in combination with propantheline bromide was studied in 75 patients with chronic duodenal ulcer. Ulcer recurrence following placebo was 75%, 39% with propantheline, 16% with SN-263, and 12% in the group which received the combination.[72] Although it seems likely that a mechanism of action of Depepsen is inhibition of pepsin, it still remains intriguing that this agent may also prevent protease digestion by binding mucus to damaged areas of the mucosa, or perhaps by altering the chemical composition of the mucus.[72]

Carbenoxolone Sodium - Carbenoxolone (Biogastrone), and the form specially prepared to release in the duodenum (Duogastrone), is a compound originating from the early observations that licorice is useful in treating gastrointestinal disorders. Carbenoxolone, the disodium salt of glycyrrhetinic acid hydrogen succinate, is believed to protect the mucosa or enhance healing by increasing mucus secretion.[73] The mechanism by which this compound acts is not understood, but it may be necessary to have gastric absorption of the compound to promote healing of gastric ulcers, and duodenal absorption for healing of ulcers located at this site.[74] It does not appear to significantly suppress gastric secretion in gastric ulcer patients, but the acid secretion in these patients was correlated with degree of healing.[75] In the same double-blind study by this group using a dose of carbenoxolone that only produced minimal side effects, significantly more ulcers healed in the treated subjects. The efficacy of Duogastrone in healing duodenal ulcers remains equivocal. Amure[76] found effective healing in a small group of ambulatory patients, while in another double blind study, also in a small group, 200 mg/day for 12 weeks produced a slight but insignificant clinical improvement.[77]

Over the years considerable interest has also been placed on variants of carbenoxolone or licorice which would be devoid of the side effects on fluid and electrolyte balance. A controlled study[78] on such

an agent (Caved-S) in gastric ulcer patients revealed significant reductions in ulcer niche and disappearance of crater in the treated group with no evidence of edema or excessive weight gain.

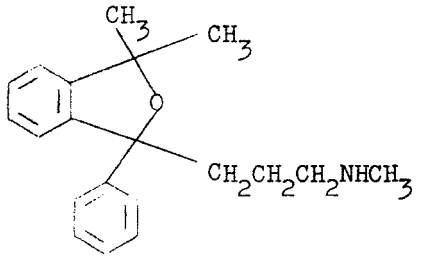
Xylamide - Introduced in Europe in 1967, Milid (Rotta; N-benzoyl-N,N-di-n-propyl-DL-isoglutamine) has been the topic of several clinical reports. In oral and parenteral doses up to 8.0 gms daily it reportedly improves the condition of the duodenal ulcer patients,[79] suppresses histamine induced secretion in duodenal ulcer patients,[80] decreases volume and acidity of gastric contents within a week, and "normalizes" secretion within three weeks.[81] It is reported to be tolerated well with no immediate or delayed side effects. In animals as well as in man the compound does not seem to suppress gastric secretion in normal secretory states.[82] Evidence suggests that the compound is not a specific "antigastrin" since it depresses histamine induced secretion at lower doses than pentagastrin stimulated acid secretion in both rats[82] and man.[83]

Miscellaneous Antisecretory Compounds

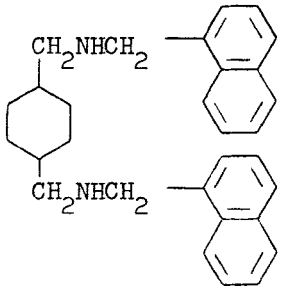
Relationship between blockade of catecholamine uptake and antisecretory activity - Following the earlier findings that imipramine inhibits basal gastric secretion in rats,[84] and also reduces acidity and relieves pain in ulcer patients,[85] studies have been published on the antisecretory activity of other compounds that block norepinephrine uptake.

DMI (desmethylimipramine) is antisecretory in the dog and rat,[86] and man.[87] DMI suppresses 2-DG induced secretion, but not carbachol induced secretion in Heidenhain pouch dogs.[86] This finding and the lack of effect on secretion produced by preganglionic electrical stimulation suggest that the site of DMI action is within the CNS.[86]

Lu 3-010 (3,3-dimethyl-1-(3-methylaminopropyl)-1-phenylphthalan), a specific blocker of catecholamine uptake devoid of anticholinergic activity, inhibited both basal and pentagastrin stimulated secretion in the rat.[88] Studies by Lippmann on Lu 3-010 and another series of compounds[89] related to N,N'-bis-(1-naphthylmethyl)-1,4-cyclohexane-bis-(methylamine) dihydrochloride (AY 9928) suggest that direct correlation between blockade of catecholamine uptake and antisecretory effect is moot.



Lu 3-010

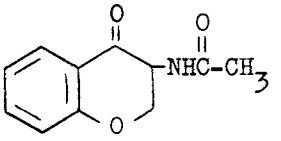


AY 9928

Lippmann also reported on the antisecretory and norepinephrine blocking effects of 2-[p-chlorophenyl-2-(pyridyl)-hydroxymethyl] imidazole maleate (Sch-12650).[90]

Suppression of gastric acid secretion through inhibition of histidine decarboxylase has been a relatively recent approach taken to find antisecretory compounds, and it does appear that these agents can decrease gastric secretion.[91-93] In the clinic, Brocresine (NSD 1055; 4-bromo-3-hydroxybenzyloxyamine) was found to suppress basal but not histamine induced gastric acid secretion in two patients with Zollinger-Ellison syndrome.[94] The decrease of secretion was accompanied by a marked relief of diarrhea. Further trials of Brocresine and other histidine decarboxylase inhibitors should be conducted to ascertain the clinical utility of this type of agent.

Biological studies indicated that 3-amino-4-chromanone hydrochloride was an active inhibitor of gastric secretion in the Shay rat preparation.[95] Of a series of 30 compounds synthesized, the most active compound [ED_{50} (sc) 2.6 mg/kg] is shown.



3-acetamido-4-chromanone

Regarding the effect of catecholamines and blocking agents on gastric secretion, conflicting results from different laboratories still are common. Differences in species used and preparation probably account for some of these discrepancies. Isoproterenol reduced acid secretion in the rat; this did not correlate with blood glucose or blood pressure effects.[96] In man, the β-agonist nylidrin (Arlidin, U.S. Vitamin) increased gastric secretion.[97] Using propranolol for β-receptor blockade, equivocal results were found in the rat,[96,98] and also in man where it either depressed secretion[97] or increased basal secretion while producing a slight shift of the pentagastrin peak of the dose response curve to the left.[99] Both α-adrenergic stimulants and antagonists seem to suppress gastric secretion in the rat.[96,98]

Thiocyanate has been recognized for many years to be a relatively potent, specific, and reversible inhibitor of gastric acid secretion. The mechanism of ^-SCN inhibition of secretion remains controversial with effects on a bicarbonate activated ATP-ase,[100] a dead-end kinetic effect on a mucosal anion exchange carrier system,[101] or cytochrome c[102] as not mutually exclusive sites of action. Studies with non-polar methyl analogs of ^-SCN and isothiocyanate cast some doubt on the possibilities that these compounds inhibit secretion via competition for carrier, or inhibition of gastric mucosal ATP-ase.[103]

Nicotine - The effects of nicotine on functions of the gastrointestinal tract are of interest both pharmacologically and clinically. Single doses of nicotine depress basal secretion in rats,[104] in normal persons and

peptic ulcer patients,[105] and in the isolated frog gastric mucosal preparation.[106] Recent studies show that in dogs nicotine inhibits contractile activity of both circular and longitudinal muscle in the upper gastrointestinal tract as well as in the descending colon.[107,108] This effect is apparently mediated by catecholamines released from adrenal glands and adrenergic nerve endings.

Agents Which Affect Motility

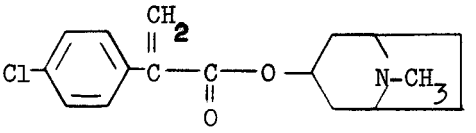
Connell and George[109] demonstrated a direct correlation between the effect of metoclopramide and the initial (pre-drug) rate of gastric emptying in man and also showed that, as in animals, metoclopramide did not affect gastric secretion. The action of metoclopramide on the motility of the stomach and intestine, both *in vivo* and *in vitro*, can be inhibited by anticholinergic drugs.[110,111] Further studies suggest that increased tone and motility produced by metoclopramide may result from both a sensitization of muscarinic receptors to acetylcholine and a blockade of non-cholinergic (e.g., tryptaminergic) receptors.[111,112]

Based on Ariens' receptor theory and agonist-antagonist concepts, some attempts have been made to design drugs which would help describe the cholinergic and other receptor sites. Studies with rigid and semi-rigid analogues of acetylcholine have shown the importance of the steric relation of the quaternary ammonium function (the minimum structural requirement for cholinomimetic activity) to the rest of the molecule.[113] Some data suggest that the receptor may consist of a large nonpolar binding surface, but do not elucidate differences in binding of the agonist and antagonists tested.[114] Hudgins and Stubbins[115] failed in an attempt to improve antagonistic action by combining structural features of agonist and antagonist in the same molecule, suggesting that receptor affinity was not improved by this approach. Studies on a tryptaminergic receptor indicated electronic rather than steric factors were significant determinants of relative activity.[116]

A technique for measuring several anticholinergic activities in the same animal preparation allows the generation of data useful in calculating "efficacy/side effect" ratios to predict clinical usefulness.[117] In order to measure drug effects on intestinal propulsion without the confounding effects on gastric emptying, Summers and others[118] have designed a technique for administering drugs intraduodenally through a chronically implanted tube.

Although structures with anticholinergic (atropine-like) activities continue to be synthesized and studied, non-anticholinergic spasmolytics also have considerable clinical potential. Mebeverine, a compound of this type, was marketed abroad several years ago; it has recently been reported to act by blocking uptake of norepinephrine as well as by a non-specific depressant effect.[119] Two new series of compounds were synthesized by removal of the alcoholic OH group from the β-carbon atom of certain atropine derivatives; these compounds retained spasmolytic action, but lacked atropinic "side effects".[120] The pharmacology of one of these

compounds, 3-tropanyl-2-(p-chlorophenyl) acrylate hydrochloride, has been reported.[121]



3-tropanyl-2-(p-chlorophenyl) acrylate

Lactulose

This synthetic disaccharide, 1,4-β-galactosido-fructose, has been in use abroad for a number of years as an effective cathartic.[122] More recently it has found potential in the treatment of hepatic encephalopathy where it may have considerable advantage over other types of available therapy.[123,124] The mechanism of action in these cases has been described as an "acid dialysis". Since lactulose is not metabolized by human disaccharidases and little is absorbed,[125] it passes to the colon where it is hydrolyzed by bacteria to lactic, acetic, and formic acids. The resultant decrease in the pH of the colonic contents has two effects: the pH gradient stimulates passage of ammonia from the plasma to the intestinal lumen, and absorbable ammonia in the lumen is converted to nonabsorbable ammonium ions which are excreted.[126,127] With the decreased ammonia content of the blood, patients have improved psychologically and behaviorally.

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Chapter 9. Antiarrhythmic Agents

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Several years have passed since antiarrhythmic agents were reviewed in this series (1965). At that time Tanz discussed the varied experimental approaches and diverse effects observed during the evaluation of new agents.

The two types of agents which have commanded most attention are β-adrenergic blocking agents and analogs possessing local anesthetic or direct nonspecific membrane action on the myocardium.

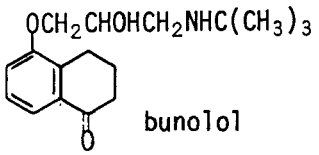
β-Adrenergic Blocking Agents - This relatively new series of antiarrhythmic agents has received considerable attention during the past few years. They antagonize the positive inotropic and chronotropic effects of catecholamines. Cardiac arrhythmias associated with excessive adrenergic stimulus[1], released endogenous catecholamines or sensitization of the heart by anesthetics[2,3] or cardiac glycosides[4] may effectively be treated by β-blockade. Some β-blockers also possess membrane or local anesthetic action and are effective against arrhythmias due to ischemia or cardiac glycoside toxicity as well. This membrane action was shown to be independent of β-blockade since resolved isomers of β-blockers possessed equal antiarrhythmic potency but unequal β-blocking action.

A recent review of β-adrenergic blocking agents has appeared, but the antiarrhythmic activities were not fully discussed[5]. The more recent compounds which were not reviewed have been included in this article.

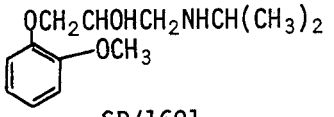
Specific β-adrenergic blocking agents are able to antagonize catecholamine actions but do not have significant local anesthetic activity to effectively reverse cardiac glycoside-induced arrhythmias. Analogs INPEA, sotolol and AH 3474 are relatively weak β-antagonists when compared with propranolol[6-9]. Pindolol[10] is reported to be at least three times more potent than propranolol. Bunolol,[11-12] has recently been reported to be at least 20-fold more potent orally than propranolol in dogs. Practolol has been described as a cardioselective β-blocker only 1/4 as potent as propranolol in the heart but 1/150 as active in the trachea of guinea pigs and may be of use therefore in asthmatics[13].

Dual-acting β-blockers are effective against a variety of cardiac arrhythmias since they possess both β-blocking and local anesthetic activity. The most prominent analogs of this group are propranolol and alprenolol[14,15]. In addition, oxyprenolol, butidrine, Ko 592 and SD/1601 have been reported as effective antiarrhythmic agents[16,17].

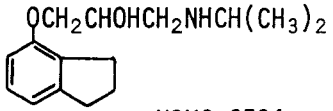
Other β-blockers reported recently to be active in the dog but not fully characterized include USVC-6524 and KL-255[18,19].



bunolol



SD/1601



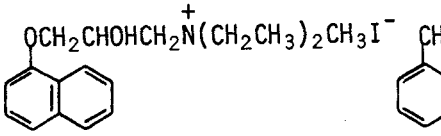
USVC-6524

A major disadvantage in the use of β-blockers is their negative inotropic and chronotropic effects on the heart. Therefore, their use in patients with incipient heart failure is contraindicated[20]. Sotolol has less depressant effects and has a better therapeutic ratio when compared clinically with most β-blocking agents[6]. Oxyprenolol and alprenolol are also less depressant upon basal heart functions possibly due to their intrinsic β-sympathomimetic effects[15,21].

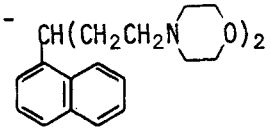
The relationship between cardiac depressant, local anesthetic and β-adrenergic blockade actions has evoked considerable interest. A common mechanism could be responsible for all actions. However, the separation of racemic alprenolol and propranolol into their optically active isomers resulted in only the levo isomers being potent β-blockers while both isomers possessed local anesthetic and cardiac depressant actions[14,15,22]. The separation of bunolol into its optical isomers resulted in a further separation of the actions of β-blockers. Bunolol is devoid of significant antiarrhythmic and local anesthetic activities. Dextro-bunolol is inactive as a β-blocker but did possess a cardio-depressant action equal to that of the levo isomer[11,12]. Thus, no correlation between local anesthetic, β-blockade or toxic cardiac depression actions exist. Separate mechanisms for the three activities probably are involved.

Local Anesthetic - Membrane Active Antiarrhythmics - Many established antiarrhythmic agents have been found to possess local anesthetic activity which parallels their effectiveness against a variety of cardiac arrhythmias[23]. These agents include quinidine, procaine amide, lidocaine and propranolol. They appear to act by depressing impulse conduction and by restoring normal automaticity to the myocardium.

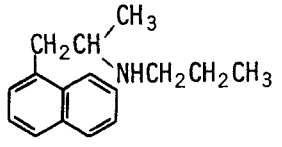
An analog (ICI 46037) structurally similar to propranolol was shown to possess local anesthetic and antiarrhythmic activity similar to propranolol in the dog. No β-blocking action and a lower level of myocardial depressant action was observed[24]. Other analogs have been reported recently having antiarrhythmic activity similar to quinidine in potency. These include 1,5-dimorpholino-3-(1-naphthyl)pentane (DA-1686)[25]; 2-propylamino-1-naphthylpropane (S-931)[26]; ethoxamine (BW 62-235)[27]; and xipranolol (BS-7977D)[28].



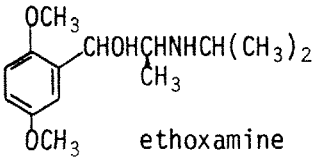
ICI 46037



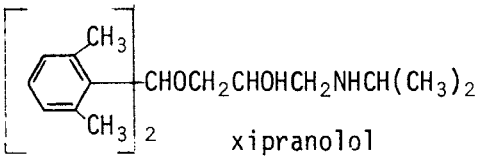
DA-1686



S-931

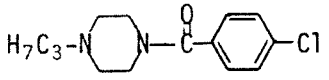


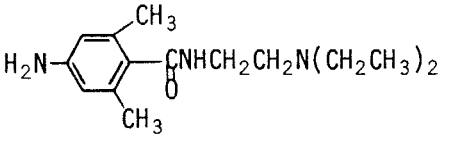
ethoxamine

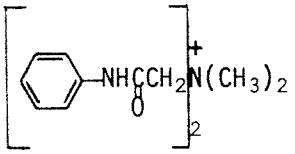


xipranolol

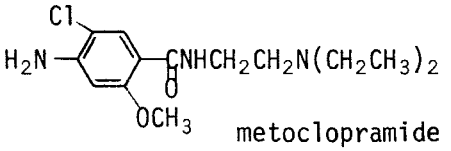
While lidocaine and procaine amide are effective antiarrhythmics, lack of oral activity[29] and toxicity has led to the development of newer analogs. The following have been reported to possess local anesthetic and antiarrhythmic activities in a number of experimentally-induced cardiac arrhythmias[30-34].



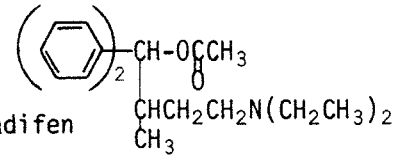




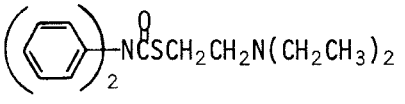
QX-572



metoclopramide



proadifen



phencarbamide

Of the analogs reported, four (ICI 46037, S-931, QX-572 and DA-1686) are claimed to possess weaker toxic depressant effects than propranolol or quinidine. One (QX-572) has a prolonged action with no cardiac depression at therapeutic dosage but has poor oral activity in man[32].

Other Antiarrhythmic Agents - Structural leads to new antiarrhythmic agents include some which have not yet been classified as to their possible mode of antiarrhythmic action. These agents include: cyproheptadine[1-methyl-4-(5-dibenzo-{a,e}-cycloheptatrienylidine)-piperidine][35]; clemizole[1-(p-chlorobenzyl)-2-(1-pyrrolidinylmethyl)-benzimidazole][36]; 10-butylphenothiazine[37]; ESP-2001 [1-ethoxy-2-(imidazolylmethyl)-naphthoate][38]; Ro-5-5340 [N,N'-iminoditrimethylene)-di-p-toluene sulfonamide][39]; 9,10-trans-5H-5-(3,4,5-trimethoxybenzamido)-2-methyldecahydroisoquinoline[40]; SU-13197 [3-(p-chlorophenyl)-2-(2-imidazolylmethyl)-1,2,3,4-tetrahydro-1-benzepine][41]; WR 9792 [4-fluoro-4'-trifluoromethylbenzophenone guanylhydrazone][42]; WR 81,844 [1-(3,4-dichlorophenyl)-3-{4-N-ethyl-3-piperidylamino}-6-methyl-2-pyrimidinyl guanidine][43]; sodium mercaptoacetate[44]; 4-ethyl-3-benzyl-5-cyclohexane-spirooxazolidin-2-one][45]; and taurine[2-aminoethanesulfonic acid][46].

Mode and Site of Action - Attempts to correlate activity with basicity, lipid solubility or molecular size revealed no simple relationship with activity[47,48]. Ritchie and Greengard[51] noted that local anesthetic activity depended upon the structure possessing an electron-rich aromatic ring. Electron withdrawing groups reduced activity. As evidenced by the diversity of structures possessing antiarrhythmic activity, no general structural requirement can be formulated. Only structures within certain series of agents such as β-adrenergic blockers can be correlated with potency and specificity.

Cardiac glycosides have both a beneficial effect of improved conduction and an ability to cause arrhythmias. An understanding of the manner in which they affect the heart function is important in the design of antiarrhythmic agents.

Cardiac glycoside toxicity and antiarrhythmic activity have been implicated in adrenergic neuronal and myogenic functions[49,50]. It has generally been assumed that digitalis and antiarrhythmic agents exert their effects directly on the myocardium. However, digitalis is also associated with adrenergic excitation at low dosage at the neural level to augment nerve function. At higher dosage, direct action on the neurones and myocardium leads to the disorganization of the synchronous stimulation and resulting arrhythmias.

Drugs which are effective against cardiac glycoside toxicity have the ability to influence neuronal structures by maintaining or restoring organized neural control. Drugs such as quinidine, propranolol, procaine amide, lidocaine and dilantin are active neurodepressants in cats at dosage levels corresponding to their antiarrhythmic potencies[49,50].

It has been demonstrated that some heart arrhythmias arise from central nervous system origin. Interruption of this central control by means of drugs or surgery restores normal heart rhythm. Supporting the view that adrenergic stimulus maintains a role in cardiac arrhythmias, it has been reported that d-propranolol and d-alprenolol are not as effective against a variety of arrhythmias when compared with the racemic forms since they lack β-adrenergic blocking activity[49,50].

Local anesthetics are able to depress or block nerve conduction at low dosage[51,52]. Davis has demonstrated a relationship between antiarrhythmic potency, local anesthesia and neuromuscular transmission[23]. The actions of antiarrhythmic agents to slow conduction can be explained by their depression of neuromuscular transmission. Slowed conduction can be explained through a retardation of the nerve impulse propagation, by increasing the electrical excitation threshold and reducing the rate of rise of the action potential[51].

Electrophysiologic Factors - Hypoxia or drug-induced net loss of intracellular myocardial K^+ causes an alteration in the transcellular ion ratio or potential. Modified automaticity and ectopic beats often result[53]. Skou[54] has reported the involvement of an energy-dependent active transport

of Na^+ and K^+ across cellular membranes promoting the transmembrane potential of cells in a number of tissues.

The effects of ouabain on a sodium and potassium dependent adenosine triphosphatase (Na,K-ATPase) were studied on heart enzymes isolated from a number of mammalian species. Ouabain inhibition in vitro was correlated with in vivo changes in heart and enzyme function at doses lower than those necessary to induce toxicity and arrhythmias[55].

In the dog heart, cardiac glycosides also caused a dose dependent release of Ca^{+2} from intracellular membranes such as the mitochondria and sarcoplasmic reticulum. This release was proportional to the magnitude of the inotropic response. The increased intracellular Na/K ratio resulting from the ouabain inhibition of Na,K-ATPase may explain the positive inotropic effects of ouabain on the basis of Na^+ stimulated release of membrane-bound intracellular Ca^{+2} [56-58]. Nakamura and Schwartz observed an in vitro increase of released Ca^{+2} when the intracellular pH was increased. Increased Na^+ or K^+ concentrations did not alter Ca^{+2} release. Intracellular acidosis due to anaerobic glycolysis during ischemia may decrease the release of calcium and reduce the contractibility of the heart muscle[59].

Further studies have shown that decreased intracellular K^+ concentration was required before ouabain induced arrhythmias in dogs. Increased Na^+ was not necessary. Local anesthetic agents such as quinidine, butidrine and procaine amide reduced K^+ loss through a membrane stabilization effect of red blood cells and not by a direct interaction with ATPase[57-59]. Andersen[63] demonstrated that lidocaine was able to alter membrane permeability to Na^+ and K^+ at therapeutic dosage. Toxic dosage levels caused a major disruption of membrane integrity which resulted in diffusion of Na^+ and K^+. The cationic form appeared to be the active species of the drug in human red blood cells.

Dilantin reversal of cardiac glycoside toxicity was well documented. Woodbury[64] has shown that dilantin augments the active transport of Na^+ and K^+ in the brain, skeletal and cardiac muscle. Its ability to reverse digitalis toxicity has been attributed to a direct action on Na,K-ATPase[65].

Agents such as cardioactive sterols inhibit Na,K-ATPase and have positive inotropic and chronotropic effects on the heart[66]. Adenosine-3',5'-cyclic monophosphate (cyclic AMP) has been shown to inhibit human gastro-intestinal mucosal Na,K-ATPase responsible for gastric secretions. This observation by Mozsik[67] may also explain the positive inotropic effects of cyclic AMP since ouabain's effects on the heart appear to be related to Na,K-ATPase inhibition.

Clinical Therapy - The use of cotherapy with existing agents represents a most significant advance in cardiac arrhythmic treatment. The combined use of two antiarrhythmic agents is effective when certain agents are too toxic or ineffective. Propranolol in combination with either quinidine, procaine amide or digitalis synergistically lowers the therapeutic dose

of each drug while reducing the incidence of toxicity[68,69].

Dilantin appears to be the most effective agent used for arrhythmias due to digitalis toxicity. When used in combination with digitalis, dilantin reduces the toxic effects while the positive inotropic action of the glycoside remains[70].

The agent of choice for the treatment of ventricular arrhythmias and those associated with myocardial infarctions is lidocaine[29,71]. Propranolol is also effective in some similar cases despite the potential hazards associated with β-blockade[72].

Bretylium tosylate is effective in treating certain pacemaker-induced arrhythmias since it has an enhancing action upon the heart conduction system[73].

It is hoped that better antiarrhythmic agents may be developed in the future by taking advantage of the more recent information concerning the electrophysiologic basis of arrhythmias.

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Chapter 10. Diuretics

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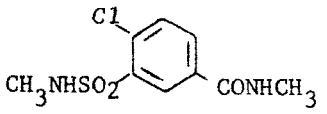
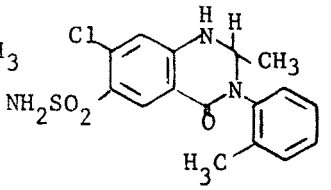
The topics to be discussed in this review include: a) clinical findings, chemistry and pharmacology of new diuretics; b) anatomical sites of diuretic action; c) biochemical mechanisms of diuresis; d) hemodynamic contributions to diuresis; and e) the antihypertensive activity of diuretics.

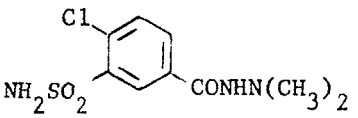
Sulfonamides - Compounds with the sulfonamide group continue to be investigated for diuretic activity. Mefruside[1] was studied in both dogs[2] and man[3] and found to closely resemble chlorothiazide as a diuretic. It was less effective initially than the thiazide, but activity persisted for 20 hr. Chronic administration resulted in both K^+ loss and uric acid retention. Mefruside inhibited carbonic anhydrase in vitro but it was not clear that this contributed to its natriuretic action. Two sulfamoylbenzamides have also received attention. Clopamide could not be distinguished from hydrochlorothiazide in dogs, either on the basis of antagonism of its action by the thiazide blocking agent, EX-4877[4], or when superimposed on a maximal hydrochlorothiazide diuresis[5]. It was a natriuretic and chloruretic drug in man, much like the thiazides, even to the extent that hypokalemia and hyperuricemia occurred during repetitive dosing[6-8]. Likewise, diapamide was comparable to the thiazides in terms of urine volume and electrolyte excretion in man[9]; elevated plasma urate and glucose accompanied chronic administration. A sulfamoylquinazolinone, metolazone, was studied in dogs and found to closely resemble hydrochlorothiazide in its effect on the kidney[10].



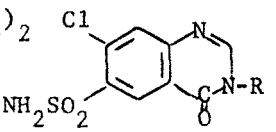
diapamide



metolazone



alipamide

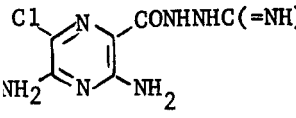


I

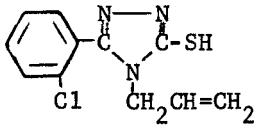
A structure activity study, done in rats in connection with the sulfamoylbenzhydrazide, alipamide[11], has led to the conclusion that a hydroxamic acid moiety could replace a hydrazide moiety without activity loss. Alkyl substitution of either sulfamoyl or juxtacarbonyl nitrogen reduced whereas alkylation of the terminal hydrazide nitrogen enhanced the activity. Efforts to apply these findings in the quinazolinone ring system (I) were only partly successful[12]. Thus, activity was enhanced when R=OH but declined when $R=NH_2$. Reduction of the 1,2 double bond not unexpectedly augmented the potency of both derivatives. Of some further interest is the fact that substitution of the sulfonamide nitrogen of hydrochlorothiazide to form the sulfonylurea resulted in a comparably potent diuretic in rats but with a better urinary Na^+/K^+ ratio[13].

A series of N-substituted 4-chloro-5-sulfamoyl-anthranilic acids increased urine volume along with Na^+, K^+ and Cl^- excretion[14]. The most active of the series (N-pyrrolylcarbonyl and N-pyrrolidinylcarbonyl) were as effective in dogs as hydrochlorothiazide and occupied the same receptors.

Pyrazines, Triazoles, Pteridines and Pyrimidines - Investigation of structure-activity relationships among analogues of amiloride has continued. Removal or replacement of amino groups on the pyrazine ring[15] or N-oxidation in the 4 position[16] generally reduced both the deoxycorticosterone (DOC) reversal and the DOC-independent saluretic activities. Likewise, substitution of carbamoyl for the amidine moiety destroyed antikaliuretic activity and such agents increased electrolyte excretion only in the rat[17]. Of somewhat greater interest is a series of pyrazinecarboxamidoguanidines from which several compounds have been selected for clinical trial[18]. In general the relation of structure to activity in this series parallels the N-amidinopyrazinecarboxamides from which amiloride was chosen. II, among the most potent of the group in reversing DOC reduction of urinary Na^+/K^+ in adrenalectomized rats and comparable to amiloride as a saliuretic in rats and man, depicts the optimum requirements. These consist of halogen, preferably Cl, at position 6, a dimethyl or unsubstituted amino group at position 5, a free amino group at position 3 and either hydrogen or an amino group on the terminal guanidine nitrogen. Most of these compounds share with amiloride a diuretic, natriuretic action independent of DOC reversal; activities could be separated to some degree in different molecules. Cyclization to form the analogous pyrazinyltriazoles often resulted in potency loss[18]. Several sulfhydryl substituted triazoles (III) were also described as non-kaliuretic diuretics[19].

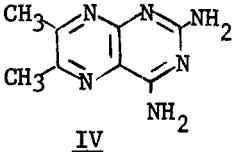


II

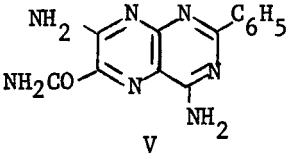


III

Weinstock et al.[20-22] have discussed relationships between chemical structures of types IV, V and VI and natriuretic and antikaliuretic activities. Many structures resembling IV were active in saline loaded rats but, of the entire series, IV alone was effective in the high mineralocorticoid, sodium deficient rat. Type V compounds frequently were natriuretic in salt loaded and salt deficient rats but also evoked kaliuresis. A similar activity profile was associated with triaminophenylpyrimido [4,5-d] pyrimidines. Natriuresis accompanied by minimal K^+ retention was greatest in the 2,4,7-triamino-6-phenylpteridine (triamterene) (VI) series. Limited substitution for or alteration of the aryl substituent in position 6, and lower alkyl substitution on the amino groups preserved quantitative and qualitative activity in many cases. These studies have led to the concept that pteridines of type VI bind to receptors at essential hydro-



IV



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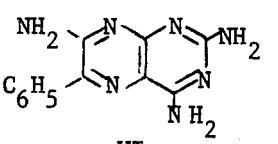
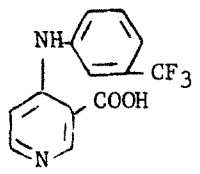


VI

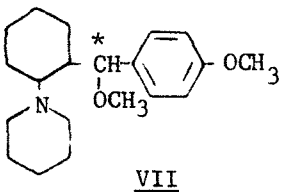
philic (N-1 or N-8) and reinforcing hydrophobic centers to block cation transport. Some variation in the intramolecular location of these centers is permissible since the 7-phenyl and 2-phenyl isomers of VI retain activity.

SC-16102 (5-ethoxyethyl-2-amino-4-azido-6-phenylpyrimidine), reported previously in terms of animal pharmacology[1], has been studied in man[23]. Urinary electrolyte and volume were increased and some potassium was lost but the duration of action was short and mild leucocytosis was observed. Site of action could not be evaluated since solute-free water clearance may have been compromised by antagonism of antidiuretic hormone (ADH).

Nicotinic acid derivatives - A new type of diuretic molecule, triflocin, promotes the excretion of as much as 30% of filtered sodium in the dog at high doses and, therefore, approaches furosemide and ethacrynic acid in maximum efficacy[24]. As with the latter drugs, sodium reabsorption was inhibited in the ascending limb of Henle's loop in animals and man but there was little effect at more distal tubular sites[24,25]. Thus, K^+ and NH_4^+ excretion increased and urinary acidity was enhanced. Glucose metabolism was not markedly altered[26]. Synthesized derivatives all were of less interest than the parent molecule[27]. 6-Aminonicotinamide also enhances sodium excretion in the rat, apparently by an action on the distal tubule[28].

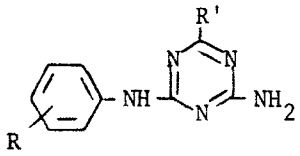


Triflocin



VII

Miscellaneous diuretics - A series of 2-amino-α-phenylcyclohexane-methanols and their corresponding ethers enhanced electrolyte and volume excretion in animals[29]. The configuration of the benzylic side chain was a determinant of activity since the erythro isomers were more effective than the threo isomers. The (+)-cis-erythro isomer of VII was the most promising compound on the basis of high oral potency, minimal kaliuresis in rats and dogs, and a favorable therapeutic index.



	R	R'
VIII	3-Cl	H
IX	4-Me	H
X	2-Cl	$SCH_2CH=CH_2$

Synthesis of a group of 2-amino-4-arylamino-6-substituted s-triazines was also described[30]. The 6-unsubstituted derivatives, VIII and IX, caused the greatest increases in urinary Na^+ excretion in rats and produced favorable Na^+/K^+ ratios. 6-Substitution with SH or OH was usually detrimental although X retained appreciable natriuretic activity.

Anatomical sites of action - The diuretic action of carbonic anhydrase inhibitors (CAI) generally has been thought to be exerted in the proximal tubule. Micropuncture evidence for reduced HCO_3^- reabsorption in this segment is reasonably firm[31,32] although evidence for reduction in Na^+ and volume reabsorption is less clear[31]. The best argument for a proximal tub-

ular site comes instead from measurements of solute-free water clearance (CH_2O) and solute-free water reabsorption (TcH_2O). During water diuresis ADH secretion from the neurohypophysis is suppressed and all tubular segments distal to the turn in the loop of Henle become nearly impermeable to water. Continued reabsorption of solute in this, the diluting segment, under these circumstances leaves solute-free water behind, and the rate of its excretion (CH_2O) quantifies the reabsorptive process. Sodium transport in this segment is not usually saturated and, therefore, can increase in response to an increasing load. Administration of CAI enhances CH_2O[33-36] which means that the quantity of sodium delivered for reabsorption to the diluting segment increases and, accordingly, proximal tubular reabsorption declines. During either hydropenia or ADH infusion, the renal collecting tubules become maximally permeable to water molecules, and the hyperosmolality of the inner medullary and papillary interstitium provides inducement for the reabsorption of solute free water (TcH_2O) in proportion to the amount of NaCl reabsorbed from the ascending limb. As would be expected from agents which increase delivery of Na^+ to the ascending limb, CAI increase TcH_2O[33]. Whether CAI also inhibit solute and volume reabsorption at more distal sites is less certain. The osmolality of early distal convolution urine is elevated by CAI[37] to suggest less sodium reabsorption by the ascending limb. However, carbonic anhydrase may not be involved in ascending limb Na^+ reabsorption since excess HCO_3^- from the proximal tubule may function as an osmotic diuretic in this segment[36].

Currently there is almost no acceptable evidence that phthalamidine, thiazide or benzamide diuretics inhibit Na^+ reabsorption in the proximal tubule. A proximal action was initially suggested on the basis of stop-flow studies but the eventual discovery of a more distal action compromised these findings[38]. Likewise, the finding that chlorthalidone inhibits Na^+ efflux from a saline droplet in the proximal tubule has not been verified by several other studies[31]. The case for a distal action of these drugs is much stronger. An increase in the distal tubular Na^+ minimum during stop-flow[39], moderate reduction in CH_2O[33,40,41], and elevated early distal convolution Na^+ and osmolal concentrations in micropuncture studies[31,37] all point to effects beyond the proximal tubule. The fact that thiazides do not reduce TcH_2O infers that these effects occur in the outer medullary or cortical portion of the loop of Henle[39,40]. The evidence is not convincing for inhibited sodium transport in the distal convolution or the collecting duct although the fact that thiazides enhance potassium secretion, which probably depends upon Na^+ transport in the late distal convolution and/or collecting duct, makes it unlikely.

Contrary to early stop-flow conclusions[42], organic mercurials have not been clearly shown to act in the proximal tubule[43] but do inhibit Na^+ reabsorption at more distal sites. The latter sites must involve at least the inner medullary and papillary portions of the ascending limb of the loop since both CH_2O and TcH_2O are usually depressed[40,44]. However, the extent of reduction in CH_2O is not as great as might be expected from the increase in osmolal clearance[44] which suggests an additional effect in the proximal tubule which is obscurred in the CH_2O measurement by simultaneous inhibition of Na^+ transport in the loop.

Ethacrynic acid (EA) exerts its principle action in the distal half of the renal tubule. This agent markedly reduces CH_2O and TcH_2O by interacting with thiazide-sensitive as well as independent sites in the diluting segment[45-48]. The renal cortico-medullary electrolyte gradient is eliminated[49] as a result of nearly total inhibition of Na^+ transport in the ascending limb. No consistent effects have been noted with EA in the proximal tubule[43]. Furosemide also blocks Na^+ reabsorption in the ascending limb, as indicated by a reduced cortico-medullary electrolyte gradient[50] and reduced CH_2O and TcH_2O, but seems able to act in the proximal tubule as well. It is not clear whether its action in the proximal segment is of consequence to its diuretic action in animals[52-55] although in man it appears definite that proximal tubular effects are exerted[48,51,56,57]. Thus, CH_2O is inhibited less by furosemide than by EA at any given rate of osmolal clearance which necessitates delivery by furosemide of more Na^+ out of the proximal tubule. It has been speculated but not proven that a proximal action of furosemide results from carbonic anhydrase inhibition[48,57].

Amiloride (and presumably triamterene) inhibits Na^+ reabsorption in the distal half of the tubule[58]. However, it neither alters the renal cortico-medullary osmotic gradient[59] nor reduces CH_2O[60,61] which suggests that its site of action is beyond the ascending limb. The HCO_3^- loss and K^+ and titratable acid retention produced by this agent[62] as well as its ability to counteract the K^+ losing effect of other diuretics[63,64] are consistent with inhibition of Na^+ for K^+ or H^+ exchange in the distal tubule. Precisely where this mechanism resides or how it works is still not clear. Net K^+ reabsorption occurs within the first third of the distal convolution but secretion is detected thereafter[65]. It has been contended that this K^+ secretion occurs by simple diffusion down an electrochemical gradient rather than by a Na^+ for K^+ or H^+ carrier exchange mechanism[66]. However, a *recent study*[67] showed that K^+ secretion, not accounted for by an electrochemical gradient and obligatorily coupled to sodium reabsorption, occurs in cortical collecting tubules. Unfortunately, the data on amiloride or triamterene do not distinguish between these possible sites of action.

Cellular mechanisms of diuretic action - The cellular mechanisms by which diuretics might act are abundant. For example, back diffusion of ions across the peritubular membrane may be enhanced, the cell's ability to generate a usable form of energy may be restricted, utilization of available high energy molecules might be inhibited, or Na^+ entry from urine to the cell may be impeded. Some evidence supports each of these possibilities.

Studies using frog skin, toad bladder and rat proximal tubules have suggested that some diuretics increase the back flux of Na^+ across the cell membrane[68]. This could result from interaction with membrane SH groups to confer additional negative charges which might create a "leak" component[69]. The swelling by kidney slices exposed to EA may reflect enhanced cell membrane permeability to Na^+[70,71]. Support also comes from the finding that renal oxygen consumption, which reflects the extent of reabsorptive Na^+ transport, was normal in kidneys of EA-treated animals in spite of profound Na^+ excretion[72]. However, heat production which presumably reflects expenditure of energy for Na^+ transport was reduced in kidneys of dogs treated

with diuretics to suggest reduced rather than inefficient Na^+ transport[73].

There are many examples of inhibited renal oxidative and anaerobic metabolism by diuretics[69-71]. Oxidative phosphorylation may be uncoupled as well[74,75]. However, these actions have not been satisfactorily demonstrated after therapeutic doses in intact animals. Lactate accumulates in the renal medulla and papilla of dogs treated with several diuretics, and this was thought to indicate augmented anaerobic glycolysis as compensation for inhibited oxidative metabolism[76]. This interpretation may not be correct, however, since oxidative metabolism facilitates, to only a small extent, Na^+ reabsorption in the renal medulla and papilla[77-80] and, furthermore, chlorothiazide, one of the effective drugs, fails to inhibit sodium reabsorption in this part of the kidney.

The possibility that diuretics inhibit utilization of available high energy molecules for Na^+ reabsorption has been extensively studied. The enzyme, Na^+-K^+ATPase, is thought to be involved in energizing renal Na^+ transport[81], but, while the diuretic action of ouabain may be explained by inhibition of this enzyme[82,83], its role in the actions of other diuretic drugs is not certain[82]. It is clear, however, that EA and furosemide somehow interfere with renal medullary energy utilization since ATP levels in this part of the kidney are elevated by these compounds[84]. Inhibition of Na^+-K^+ATPase could produce this buildup. On the other hand, such effects certainly would not result from reduced oxidative or anaerobic metabolism.

Hemodynamic contributions to diuresis - Almost certainly, conventional diuretics prevent renal reabsorption of electrolytes by interacting with components of tubular cells. However, hemodynamic mechanisms may also be involved. Ethacrynic acid[85,86] and furosemide[86,87] reduce renal vascular resistance (RVR) and, thereby, enhance renal blood flow (RBF). The increase in RBF is proportional to the level of RVR when these drugs are administered[88,89] and occurs primarily in the middle renal cortex[90]. These agents are not vasodilators in the conventional sense since they do not increase blood flow in other vascular beds nor at pressures in the kidney below the autoregulatory range[89]. Moreover, diuresis itself does not explain the effect since thiazides[87,91] or mercurials[87] reduce rather than increase RBF. While the natriuretic effect of ethacrynic acid and furosemide is probably supplemented by the change in RBF, its contribution appears small. Thus, increased RBF can be demonstrated for the duration of natriuresis if urine volume is replaced[92] but in the more normal circumstance in which a fluid deficit develops, natriuresis substantially outlasts increased blood flow[93]. This is also evident from a study in which RBF was held constant after administration of WY-5256, a pteridine carboxamide diuretic[94]. This drug normally produced a moderate natriuresis, much of which persisted in the absence of a hemodynamic change.

Most studies of mechanisms by which reduced RVR leads to increased sodium excretion have involved agents which have no independent natriuretic activity. Dopamine, for example, produces natriuresis by interacting with dopamine specific receptors to dilate the renal vasculature[95]. Likewise, acetylcholine, infused into the renal artery, dilates kidney blood vessels

and increases electrolyte excretion[96]. Other agents such as hydrazine[97] and the natural products, prostaglandin[98-100] and bradykinin[101], behave similarly. The essentiality of increased RBF to the natriuresis has been clearly demonstrated with both acetylcholine[102] and bradykinin[101]. The anatomical segment upon which at least acetylcholine acts is the proximal convoluted tubule[103,104]. The precise mechanism seems to involve increased hydrostatic pressure[105,106] and/or reduced protein osmotic pressure[107,108] in peritubular capillaries. The hydrostatic pressure increment develops from reduced resistance beyond interlobular arteries, while the decline in colloidal osmotic pressure is a product of enhanced plasma flow through the glomerulus in the absence of increased ultrafiltration. In either case the tubular reabsorptive process is slowed because fluid and electrolyte is less readily mobilized back into the blood stream.

Antihypertensive effects - The ability of natriuretic drugs to reduce elevated blood pressure has been recognized for many years although the mechanism remains in doubt. Early studies disclosed a reduction in plasma volume (PV), secondary to the diuresis and coincident with the fall in blood pressure[109,110], which was accompanied by sufficient reduction in cardiac output (CO) to explain the hypotensive action[110]. However, after sustained treatment, PV appeared replenished[109,111] and CO normal[112] which suggested that diuretics eventually reduce blood flow resistance in the vasculature. In spite of much effort, no firm evidence of a direct vascular effect has come forth in subsequent years. Moreover, evidence favoring the diminished CO concept is again mounting on the basis of several long-term studies which disclosed persistently decreased PV[113-116]. Even if PV recovers, the concept retains merit since barostatic accommodation may occur and result in reduced capacitance vessel tonus which would diminish effective PV and, therefore, CO. This is suggested by the over-recovery of PV when long term diuretic treatment is stopped[109]. Complicating this reasoning, however, is the finding that the acute hypotensive action of furosemide or hydrochlorothiazide is prevented by maintenance of body sodium but not of PV[117].

Whether volume or sodium depletion is more important, and providing there is no direct vascular action, agents with comparable salt losing capability should exert equivalent effects on blood pressure. By and large, this seems to be true. For example, chlorexolone is equivalent to hydrochlorothiazide in its effect on both sodium balance and blood pressure[118]. Similarly, furosemide[119] and ethacrynic acid[120], at doses effecting comparable weight loss, were indistinguishable from thiazides in their antihypertensive effect. Of some surprise, however, is the conclusion that highly efficacious furosemide and ethacrynic acid are less antihypertensive than the thiazides[121,122], although it is not clear whether comparable natriuresis was achieved. The aldosterone antagonist, spironolactone, is also active chronically; its efficacy rivals hydrochlorothiazide in terms of blood pressure and PV reduction[123,124]. Conversely, amiloride, which retains K^+ like spironolactone and also evokes net sodium loss in man, appears less effective than thiazides as an antihypertensive agent[125-129].

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Section 3 - Chemotherapeutic Agents

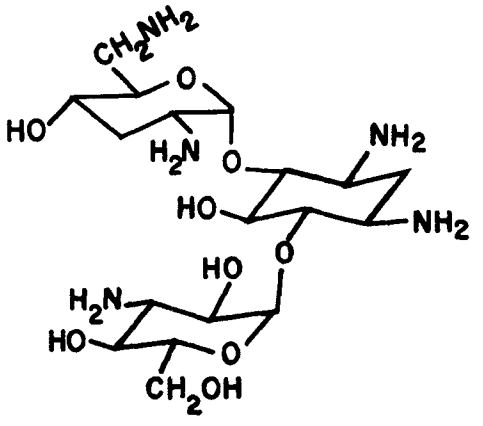
Editor: Lloyd H. Conover, Pfizer Inc., Groton, Connecticut

Chapter 11. Antibiotics

Kenneth Butler and Frank Sciavolino, Pfizer Inc., Groton, Connecticut

General - A general review of the clinical applications and side effects of antibiotics has been published.[1] Retrospective analyses have appeared which deal with the changing ecology of bacterial infections as related to antibacterial therapy,[2] and with the discovery of new and improved antibiotic substances from microbiological sources.[3] These surveys span the years since the introduction of the first effective sulfonamide up to present times. A symposium on the problems of drug resistant pathogenic bacteria was held in October.[4] A textbook on transferable drug resistance was published.[5] Structure-activity relationships in the tetracycline series were reviewed;[6] this article, which contains much previously unpublished data, provides a useful counterpart to an earlier review of the chemistry of tetracyclines.[7] The antimicrobial activities of semi-synthetic penicillins,[8a] cephalosporins,[8b] and coumermycins[8c] were also the subjects of extensive review articles. Other general reviews of major classes of antibiotics including macrolides,[9a] aminoglycosides[9b] and miscellaneous antibiotics[9c] appeared. Two massive volumes containing the proceedings of the 6th International Congress of Chemotherapy were published.[10]

Aminoglycosides - The structure (1)[11] and a preliminary pharmacological evaluation[12] of pseudomonas-active aminoglycoside antibiotic nebramycin factor 6 were reported. Factor 6 inhibited all Gram-negative species of bacteria studied at 2 mcg/ml or less and was reported to be more active than gentamicin against 48 Pseudomonas aeruginosa strains. Peak serum



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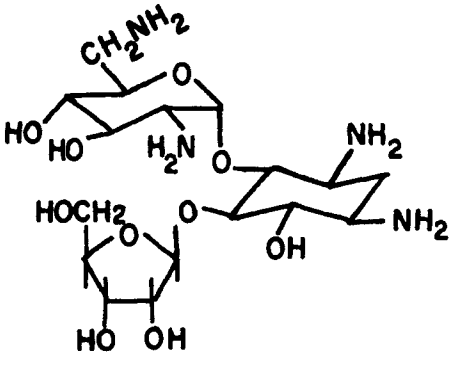

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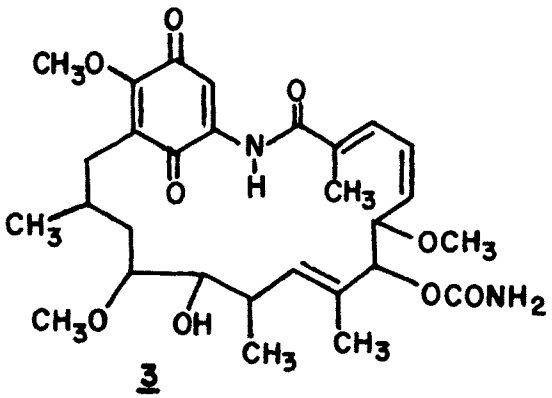
levels of 2.8 mcg/ml were observed within 1 hour following administration of 75 mg of factor 6 intramuscularly. Antibiotic 6640 (sisomicin) was announced as a new aminoglycoside structurally related to gentamicin C_{1A}; it has approximately 5 times the therapeutic activity of gentamicin in mice and twice the acute toxicity.[13-15] SF-733 (ribostamycin) (2), a chemically novel 2-deoxystreptamine aminoglycoside was isolated,[16] characterized[17] and synthesized.[18] Gentamicin resistance among clinical isolates of Pseudomonas aeruginosa was reported to arise principally from changes in sensitivity at the ribosome level (chromosomal changes).[19] High level transferable resistance to gentamicin was observed in strains of Klebsiella pneumoniae, Escherichia coli and Enterbacter aerogenes carrying R-factors.[20]

Ansamycins (Ansa-macrolides) - Results of a world-wide evaluation of the toxicity and clinical effectiveness of rifampicin have been reported.[21] Clinical and bacteriological cures generally fulfilled the expectations based on in vitro studies. Original pathogens were eliminated in 70 - 93% of infections due to staphylococci, pneumococci and gonococci, and in 45 - 58% of cases due to streptococci and Gram-negative bacteria. Rifampicin is recommended as a primary treatment for tuberculosis.[22] More than 82% of recent cases, and more than 54% of chronic patients showed improvement on rifampicin therapy.[21,22] When used in combination with other antitubercular agents, the clinical cure rate was 82 - 96.4% for chronic cases;[21,23] emergence of resistant strains is reported to be low.

In vitro tests for susceptibility to rifampicin of 476 recent hospital isolates[24] show all Neisseria gonorrhoeae and Hemophilus influenzae were inhibited by <1 mcg/ml; all but 3 of 125 strains of Staphylococcus aureus (86% were penicillin G and/or methicillin resistant) were inhibited by 0.02 mcg/ml.

Reports of the effects of rifampicin on RNA synthesis in E. coli[25] and rat liver nuclei[25,26] have appeared; rifampicin inhibits the initiation of new RNA chains.

Geldanamycin (3) is the first member of a new benzoquinone subgroup of the ansamycins.[27,28]



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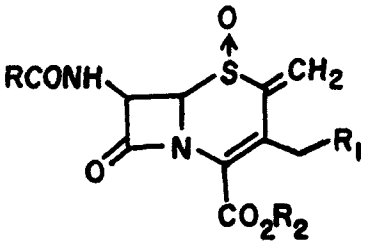
β-Lactam Antibiotics - A series of publications described the intricate rearrangement of penicillin sulfoxides to cephalosporins.[29-36] The existence of a penicillin sulfoxide-sulfenic acid equilibrium was shown by incorporation of deuterium in penicillin V sulfoxide,[37] and by trapping of the sulfenic acids.[38] Cephalosporin sulfoxide esters react with formaldehyde to produce 2-methylene derivatives (4) which have been reduced to the biologically active 2-methyl cephalosporanic acids.[39] The 2-methylene derivatives (4) were further modified to provide 2-thiomethyl (5) and 2-thiomethylene (6) adducts.[40]



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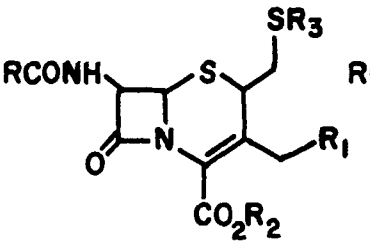

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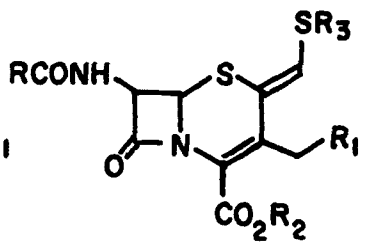

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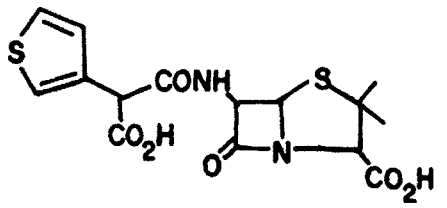
An efficient chemical conversion of benzylpenicillin to 6-APA has been described.[41]

Detailed X-ray crystallographic data of certain cephalosporin antibiotics was applied to obtaining insights into biological activity in this series.[42] The C_6-epimer (trans-isomer) of benzylpenicillin was found to be very stable to penicillinases prepared from S. aureus and E. coli carrying an ampicillin-resistant R-factor, and was a powerful inducer of penicillinase synthesis in S. aureus and cephalosporinase synthesis in P. vulgaris.[43]

Carbenicillin became available for general clinical usage in the U. S. during the second half of 1970. The clinical utility of this parenteral antibiotic has been demonstrated for Gram-negative infections especially those caused by Proteus and Pseudomonas species. The chemistry and clinical profile of carbenicillin were reported.[44,45]

Among new semi-synthetic penicillins, BRL 2288 (7) is reported to have a similar in vitro spectrum to carbenicillin but is more active against Pseudomonas species; 89% of strains were inhibited by 100 μg/ml.[46] Pivampicillin (8), an oral pro-drug form of ampicillin, provides 3 - 5 times the blood levels and 5 - 10 times the tissue levels of an equivalent dose of ampicillin.[47] BRL-2333 (9) has the same in vitro potency and spectrum as ampicillin, except that it is slightly less active vs. H. influenzae.[48] Human serum and urine peak levels are 2 - 3 times those

obtained with ampicillin; in animal protection tests it proved superior to ampicillin *via* oral and parenteral routes.[49,50] BL-P 1654 (10) is a new broad spectrum penicillin, active against ampicillin sensitive organisms and some *Klebsiella* and *Pseudomonas* strains; MIC's are very dependent upon the culture medium and are adversely affected by calcium and magnesium ions.[51,52] Flucloxacillin is better absorbed than cloxacillin (peak blood levels = 14.7 μg/ml), and is less protein bound than dicloxacillin.[53] It is clinically useful against penicillin-resistant staphylococci,[54] and may prove to be as effective as cloxacillin,[55] but at much smaller dosage regimens.



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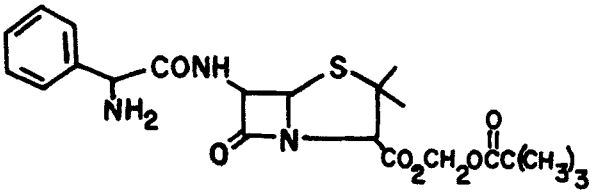

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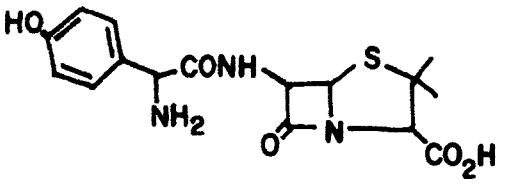

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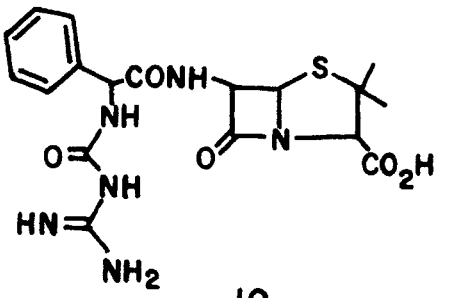


10

Cephalexin has proved to be valuable and safe, particularly for urinary and respiratory infections.[56-60] The nephrotoxic properties of cephaloridine have been reviewed.[61,62] Cefazolin is reported to be more potent than other cephalosporins against Gram-negative rods; i.m. injection provides 2 - 3 times the serum levels obtainable with cephaloridine, and it is rapidly excreted unchanged in urine.[63-66]

Lincomycin/Clindamycin - Total syntheses of lincomycin were reported from two laboratories.[67,68] A chemically novel route to 1-demethyllincomycin *via* oxidative N-dealkylation of lincomycin was described.[69] The clinical profiles of lincomycin and clindamycin were reviewed.[70]

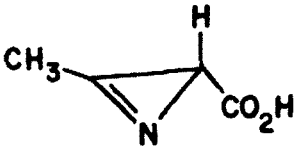
Macrolides - The X-ray structure of kromycin, the aglycone of pikromycin, has been determined,[71] confirming the 14-membered ring nature of the parent antibiotic. The total absolute configuration of methymycin was established,[72] and partial absolute configurations for neomethymycin and narbomycin were assigned.[73] The structure of lankamycin has been revised.[74] The sugar substitution pattern is reversed from the previously proposed structure;[75] D-chalose is bound at C-5 and 4-O-acetyl-L-arcanose is bound at C-3. The new structure is in accord with the suggested common biosynthetic origin of the various macrolide antibiotics. A structure has been proposed for B-58941 which contains the novel macrolide sugar 2,3,6-trideoxyhexopyranos-4-ulose.[76] Josamycin has been found to be identical with leucomycin A_3.[77] The crystal and molecular structure of demycarosyl leucomycin A_3 was determined.[78] A report on the chemistry of erythromycylamine appeared,[79] differing in several aspects from results previously reported from other laboratories. The isolation and structure of 5,6-dideoxy-5-oxoerythronolide, a shunt metabolite of erythromycin biosynthesis was described.[80]

Peptide Antibiotics - Janiemycin, a new peptide antibiotic was reported to be active against Gram-positive infections in mice at dosage levels equal to those required with penicillin G.[81] Syntheses of cyclic peptides related to gramicidin S,[82] polymyxin D_1[83] and polymyxin M[84,85] were reported. The gramicidin analogs were devoid of biological activity. Synthesis of the polymyxin D_1 cycloheptapeptide confirmed the previously proposed structure; synthetic material was comparable in efficacy with the natural antibiotic against E. coli and K. pneumoniae in vitro.[83]

Tetracyclines - Treatment of gonorrhea with a single 250 mg dose of minocycline in 250 male patients gave an 80 - 92% incidence of cure.[86] Doxycycline has been studied in cases of severe renal impairment; no accumulation of antibiotic occurred in contrast with other tetracyclines.[87,88] The structure of chelocardin, a tetracycline first described in 1962, was determined.[89]

Miscellaneous - Negamycin, a new broad-spectrum antibiotic of unknown structural class, active against Pseudomonas organisms and exhibiting high urine concentrations was announced,[90] and its mechanism of action studied.[91,92] --Structures were assigned to the optically active lipids, diumycinol, isodiumycinol and diumycene derived from the phosphorus containing diumycin antibiotics.[93] --Macarbomycin, a swine growth promotant structurally related to the diumycins, was reported to preferentially inhibit E. coli strains carrying episomes such as F, R and T factors.[94] --Isolation details,[95] a new synthesis,[96] and chemical transformations[97] of phosphonomycin were published. --Azirinomycin (11)

was reported as the first example of a natural product containing an



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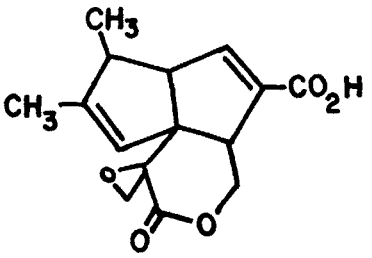

12

azirine ring; although active against Gram-positive and Gram-negative bacteria in vitro, it did not afford protection against lethal bacterial infections.[96] Pentalenolactone, an antibiotic first reported in 1956 as PA-132, was assigned the novel tricyclic lactone structure 12 by X-ray diffraction studies.[99]

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Chapter 12. Synthetic Antibacterial Agents

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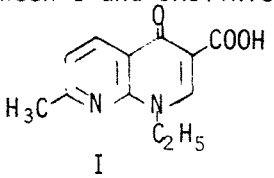
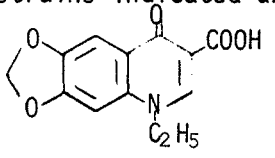
Only a few reports of novel, clinically useful drugs appeared during 1970. The major synthetic efforts were directed toward modifications of existing drugs. Advances in the development of synthetic antibacterial agents, synthetic tuberculostatics and disinfectants were reviewed[1]. The proceedings of the 6th International Congress of Chemotherapy - Tokyo 1969, had several dozen papers dealing with all aspects of antibacterial chemotherapy[2]. The third edition of A. Burger's "Medicinal Chemistry" was published in 1970[3] and chapters on drug design, chemotherapy, sulfonamides, antimycobacterial agents and antiseptics make this an excellent introduction to the field of synthetic antibacterial agents.

A 10 year study with E. coli and the Klebsiella-Enterobacter group showed that the incidence of resistance to a particular drug decreased as the widespread use of the drug decreased[4]. The indiscriminate use of drugs is indicated as the cause for the increase in bacteremia seen in hospitals[5], due possibly to an upsurge in resistant strains. In a somewhat radical approach, the control of infections due to the Klebsiella-Enterobacter group in a neurosurgical intensive care unit, by withdrawal of all anti-bacterial agents was described[6].

Quinolone Antibacterial Agents - Nalidixic acid (I) suspension has been successful in the treatment of urinary tract infections in children[7] and intravenous use has been recommended for urogenital sepsis[8]. Several cases of photosensitization due to I have been reported[9]. A comparison between I and oxolinic acid II in 40 meningococcal strains indicated an



I



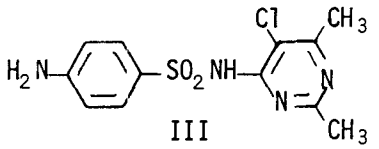
II

average MIC of 0.68 µg/ml for I and 0.14 µg/ml for II[10]. Both I and II have been found to be more active than carbenicillin or gentamicin in the treatment of hospital patients with Providence strain infections[11]. Oxolinic acid has been found effective in the treatment of recurrent urinary tract infections[12].

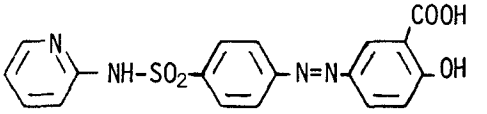
Sulfonamides - The synergistic combination of sulfamethoxazole with trimethoprim has emerged as a first choice drug in the treatment of salmonellosis[13,14], chronic pyelonephritis[15], non-specific and chronic urinary tract infections[16], and upper respiratory tract infections, especially chronic bronchitis[17,18]. Favorable results were also achieved in the treatment of purulent angina, bacterial skin infections[19], staphylococcal osteomyelitis[20], and endocarditis due to E. coli[21] as well as a variety

of other problems. Side effects encountered include allergic rashes, abdominal pain, vomiting, convulsions[17], and a fall in thrombocyte and reticulocyte counts[16,22].

A new broad spectrum sulfonamide, sulfaclomide III, was reported to be relatively non-toxic (LD_{50}, p.o., >6g/kg., in rodents) and to give higher effective serum levels than other sulfonamides. A daily maintainance dose of 2.5 mg/kg and no extra fluid intake nor alkalization is

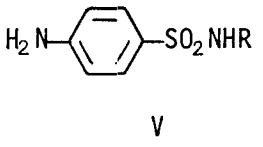


III

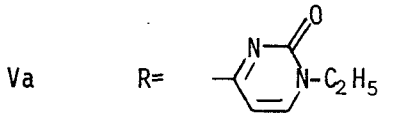


IV

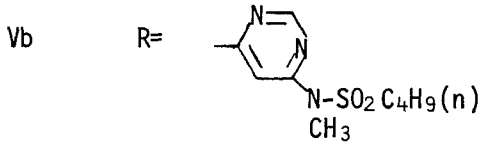
recommended[23]. Urinary tract infections in children due to sensitive E. coli, are treated with sulfadiimidine or sulfafurazole[24]. Sulfonamide resistant E. coli were found to have replaced previously sensitive organisms in children with refractory E. coli infections[25]. This was attributed to previous antibiotic treatment which altered the flora in the large intestine. Salicylazosulfapyridine IV has been found to be effective in the treatment of ulcerative colitis, with no change in the relapse rate in a study based on two consecutive 5 yr. periods[26]. In chronic, intermittent, ulcerative recto-colitis, the combination IV and steroids is considered the therapy of choice[27]. A promising new lead, N-sulfanil-1-ethylcytosine (Va), was 3-10 times more potent in vivo than sulfisoxazole, sulfisomidine, and sulfachlorpyridazine[28]. It was rapidly absorbed and excreted almost unchanged. Due to its high solubility it should not produce crystalluria. In vivo activity was also reported for Vb, which had only 10% of the potency of sulfamonomethoxine, but was more potent than



V



Va

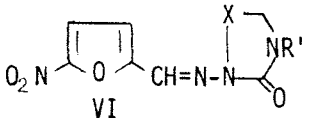


Vb

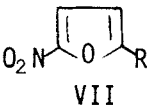
sulfisoxazole against staphylococcal infections[29]. A correlation was found between the log of the association constant and molecular weight of sulfonamides and a relationship between these two parameters and activity against E. coli was derived[30]. A linear relation has been observed between the pKa values of sulfonamides and the prostatic fluid/plasma concentration ratios in dogs[31]. The metabolic fate of sulfonamides has been reviewed[32].

Nitrofuran and Related Antibacterials - Several clinical studies with nitrofurantoin VI (X=C=O, R^1=H) indicate that it is effective in treatment of

urinary tract infections[33,34]. One of these papers[34], however, recommends it for long term prophylaxis rather than therapeutic use. Pulmonary reactions to VI have been reported[35]. The hydroxymethyl analog VI (R^1= CH_2OH) was also found to be effective in clinical urinary tract infections[36]. Analogs of Nifuradene VI (X=CH_2, R^1=H) were prepared[37] in which

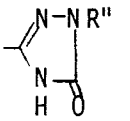


VI

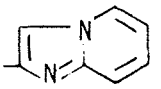


VII

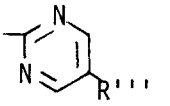
VIIa R=



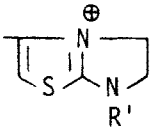
VIIb R=



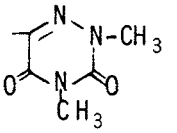
VIIc R=



VIId R=



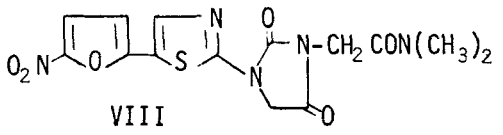
VIIe R=



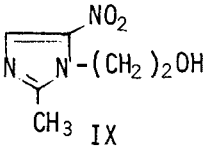
VIIf R=-CH=N-CH$_3$

R^1 was substituted. The in vitro and in vivo potencies indicated that best activity is obtained with R'=CH_2OH or -$(CH_2)_2OH$ (MIC vs E. coli = 0.8 µg/ml and 3.1 µg/ml, respectively). Some 1,2,4-triazolinones of type VIIa were prepared[38] and the analog wherein R"=CH_2CH_2OH was found to be more potent in vitro than nitrofurazone vs E. coli, P. vulgaris, S. typhosa and Ps. aeruginosa. A group of 5-nitro-2-furyl derivatives of indolizine, imidazo[1,2-a]pyrimidine and imidazo[2,1-b]thiazole were prepared[39]. The most active was VIIb, with an MIC ~ 100 µg/ml vs E. coli. Nitrofuryl pyrimidines of type VIIc were prepared[40] and found to be in vitro active. The most active had MIC values of <2 µg/ml against E. coli, S. aureus and K. pneumoniae. Ten analogs of furazolium VIId (R'=H) were prepared[41] with the best (R'=CH_2-C$_6$H$_4$-Cl) having ca. 1/2 the activity of the parent compound. Some triazinediones were active in vivo against E. coli in mice[42]. The most active compound VIIe had an MIC of 0.4 µg/ml vs E. coli. Nitrones had slight to moderate activity in vitro. The most potent, VIIf had an ED_{50} of ~ 45 mg/kg vs Salmonella in mice[43]. In a group consisting of 1-[4-(5-nitro-2-furyl)-2-thiazolyl]hydantoins, hydrouracils and related compounds, the best in vivo active compound was VIII. It had an ED_{50} of 65 mg/kg, p.o. and 110 mg/kg, s.c. vs S. aureus in mice[44].

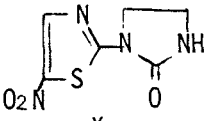
The antibacterial spectrum of metronidazole IX was studied in 51 species (400 strains) and was found[45] to be most effective vs anaerobic species (MIC range of 0.03-8 µg/ml). Niridazole X was active against many Salmonella strains in vitro[46] and was active against S. typhimurium in mice. In a group of 6 nitrobenzofurans, 3,7-dinitro-2-methylbenzofuran was the most potent and had a similar antibacterial activity and mode of



VIII

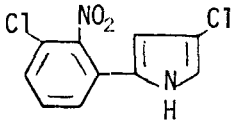


IX

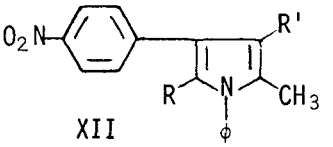


X

action to nitrofurazone[47]. Arylamino-5-aminomethyl-1,3,4-thiadiazole derivatives had activity against both gram-positive and gram-negative organisms[48]. Nitro-1,3,4-thiadiazole-2-carboxaldehyde derivatives[49] and a group of 1-substituted-2-nitrobenzimidazoles[50] were also reported to have <u>in vitro</u> activity. Synthetic pyrrolnitrin (XI) analogs of type XII



XI

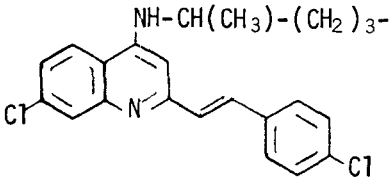


XII

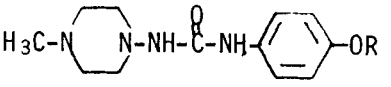
were active <u>in vitro</u> against a broad spectrum of organisms[51]. Des-nitro analogs of XI had a broader spectrum and were more active <u>in vitro</u> than the parent compound[52].

<u>Antitubercular Agents</u> - A survey of the history and control of tuberculosis and a review of present day therapy appeared[53]. An entire volume of Antibiotica et Chemotherapia[54] was devoted to the experimental and clinical evaluation of tuberculostatics. It deals extensively with capreomycin, thiocarlide, ethambutol and rifampicin. The combination of INH, rifampicin and ethambutol is expected to prove more effective than all previous forms of treatment[55], for the initial stages of TB as well as for relapses and resistant cases. Prothionamide was studied in combination therapy and gave results comparable to ethionamide, without gastrointestinal disturbances[56]. Liver toxicity was however greater and occurs without early symptoms[57]. Synthetic antitubercular preparations[58] and the modern chemotherapy of tuberculosis are reviewed[59]. In contrast to the usually optimistic reports, a study to determine the effect of recent advances in tuberculosis treatment, and comparing two five-year periods, found no marked improvement in prognosis. A comparison of the results of surgery and drug therapy showed that the former was superior in the young and middle aged[60].

The <u>in vivo</u> active compounds included SQ 18571 (XIII) which was about twice as active as its o-chloro analog (aminoquinol) and 1/5 as active as isoniazid. Large single weekly doses were as effective as small daily doses and no resistance developed[61]. Compounds of type XIV were found to



XIII

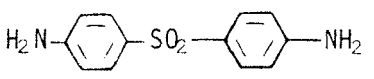


XIV

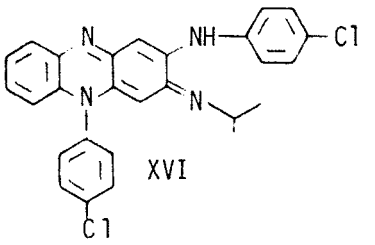
exhibit potent activity against M. tuberculosis. They were well tolerated and showed stronger activity than ethoxide in mice[62].

Compounds tested and found active in vitro against M. tuberculosis include: 5-arylamino-3-phenylthiazolidin-2,4-diones[63], 2,3-diaryl-5-arylazo-4-thiazolidinethione-1,1-dioxides[64], N-substituted benzisothiazolin-3-thiones[65], 4-(indol-3-yl)-imidazoles[66], indol-3-ylcarboxylic acids and hydrazides[67], 3-halophenylazoindoles[68], 3,4-dihydropyrido[2,3-d]pyridazin-1[2H]-one[69], Schiff bases from isoniazid and substituted benzaldehydes[70], 5-n-butylpyridine-2-carboxylic acid hydrazide[71], 4-thiosemicarbazido-2-[(5-nitrofuryl)vinyl]quinolones and butadiene analogs[72], 3-thio-4(3H)quinazolone derivatives[73], and N-(o-tolyl)-N^1-(2-benzothiazolyl)-N"-alkylquanidines[74].

Leprosy - In a survey of current treatment, 4,4'-diaminodiphenylsulfone (DDS) (XV) remains the drug of choice for all forms of leprosy[75]. Combined therapy utilizing DDS and the polyoxyethylene ether, Macrocyclon, was not superior to DDS alone. Thiambutosine and carbanylimide gave favorable results, however, cost and resistance development render them "second choice" drugs. The value of long lasting sulfonamides remains a controversial issue[76]. Thalidomide gives remission to the lepra reaction and relief from neuritic pain[77]. Clofazimine (XVI) appears to be a most effective antilepral agent in animal experiments[76,77]. Clinical trials in Africa gave excellent results[76] and a successful controlled double blind



XV



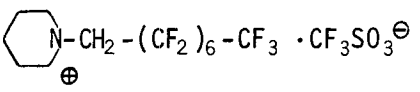
XVI

clinical study has been published[78]. Data on antimicrobial activity[77], acute and chronic toxicity and reproductive toxicology have been reported[79]. In addition to its antimicrobial effects, XVI may inhibit or prevent lepral symptoms and may reduce steroid or thalidomide requirements[76]. The most serious side effect is a dose dependent intense skin pigmentation[80].

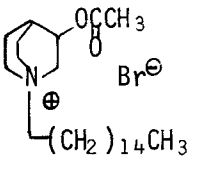
Antiseptics and Topical Agents - Phenylmercuric acetate and nitromersol potentiated the in vitro and topical antimicrobial effects of hexachlorophene and dichlorophene[81]. Chlorhexidine has been recommended as a disinfectant for surgical instruments[82]. It has potent in vitro activity and is stable and non-irritating. The antiseptic properties of benzothiazolyl guanidines were studied and potent in vitro activity was found[83]. Twenty-eight 8-hydroxyquinoline esters and their chelates were synthesized and found active against E. coli and S. aureus in vitro[84]. The esters were more active than the chelates. Some 7-amino derivatives of 8-hydroxyquinoline were found[85] to have moderate broad spectrum activity. Thiol analogs were found to be inactive and only some tin salts had any

activity[86].

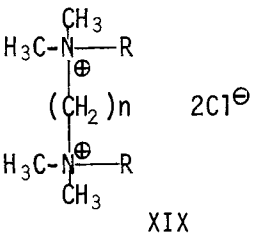
Quite a few papers dealt with quaternary salts. The fluorinated quaternary XVII was found comparable to cetylpyridinium chloride but less irritating and less toxic[87]. A group of side chain quaternized phenothiazines had in vitro activity. The most active had MIC values of 1 μg/ml vs.



XVII



XVIII



XIX

E. coli and S. aureus[88]. Some quinuclidinium compounds[89] were quite active in vitro with the most active XVIII having MIC values of 0.4 μg/ml for S. aureus and B. subtilis. A group of 68 N,N-dimethyl-N-alkyl-2-aryloxyethyl ammonium bromides were prepared[90] and screened vs 8 common infectious organisms, and the best had alkyl groups of from 8-12 carbon atoms, with MIC values as low as 0.5 μg/ml. Bis-quaternary salts of ethylene and hexamethylene diamine of type XIX were active in vitro[91]. The most active had R=$C_{10}H_{21}$ and the chloride had greater activity than the corresponding iodide. Another paper dealt with the structure-activity relationships of compounds of type XIX[92]. Various physical properties such as pH, wettability, viscosity, surface tension, etc. were related to activity.

Miscellaneous In Vitro Active Compounds - The following types of compounds exhibited in vitro activity but were either inactive in vivo or no in vivo data were supplied.

Quinazolines: 2,4-diamino[93], anilino[94] 8-hydroxy analogs[95], 4-spiro analogs[96] and quinazoline-5,8-diones[97]; 3-hydrazides and thiosemicarbazides of isatin-1-acetic acid[98], 3-acylhydrazones of 1-dialkylaminoalkyl isatins[99], indolophenazines[100], 4- and 7-hydroxycoumarins and derivatives[101], amides and hydrazides of 4-dimethylaminosalicylic acid[102], substituted N-phenylanthranilic acid hydrazides[103], substituted 1,3-distyryl-4,6-dinitrobenzenes[104], substituted β-nitro styrenes[105], synthetic aliphatic polyamines related to spermine[106], guanidino derivatives of dehydroabietylamine[107], bis quaternary ammonium salts of cyclohexane[108], 4-aza-22-oxa-5α-cholestane[109], mono- and diazaphenanthrenes[110], piperidine and tetrahydroquinoline analogs of tetrahydrofolic acid[111], quinoline-N-oxides and hydroxamic acids[112,113], 5-arylazo-6-substituted aminopyrimidines[114], fluorinated deoxy uridine derivatives[115], N-hydroxythioureas[116], hexachlorocyclopentadiene adducts of unsaturated amides[117], 1,4-naphthoquinones and halogenated derivatives[118], 2-mercapto benzothiazole salts and derivatives[119], acetophenones, chalcones and benzylidene flavanones[120], and alkoxythioformyl disulfides[121].

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Chapter 13. Antiviral Agents

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Introduction - This review will concern itself in large measure with a discussion of interferon as an antiviral agent and with drugs that cause its formation and release. Although this area is by no means thoroughly understood, a great deal of research has been devoted during the past year to this aspect of antiviral chemotherapy. There were several reviews written concerning interferon and other antiviral drugs[1-9]. A survey of antiviral drugs for 1969 was also presented[10]. Outside of the interferon area no major discoveries were disclosed, and no drugs reached the marketplace or were reportedly newly introduced into man.

Interferon - This continued to be an extremely active area both in the laboratory and in human studies. The role played by interferon in the course of natural varicella infection was studied[11] in human patients with and without impairment of host-defense mechanisms. In infected patients with normal defense mechanisms, interferon titres present in cutaneous vesicles were initially high, and appeared to prevent virus dissemination and allow rapid recovery. On the other hand, in patients with Hodgkins disease, lymphomas and leukemias where there is an impairment of host-defense mechanisms, low titres of cutaneous interferon were initially present, and viral dissemination was rapid and in some cases led to death. In those cases which were resolved favorably the remission followed the late appearance of high interferon titres.

In a study with human volunteers challenged with A_2 Asian influenza, a correlation between virus shedding, clinical illness, interferon titres and antibody was attempted. It was demonstrated[12] that the development of clinical symptoms paralleled the rise in interferon titres and these symptoms subsided as the interferon titres reached a maximum. Although it was felt that interferon probably limited the spread of the disease and caused some clinical improvement, it was concluded that it was less effective than antibody in decreasing and eliminating the shedding of virus and final recovery from disease.

In animal experiments[13] it has been found that macrophages stimulated to produce interferon by a non-replicating virus (chikungunya virus) can be transferred to mice already infected with encephalomyocarditis (EMC) or Semliki Forest (SFV) virus and exhibiting clinical symptoms, and will afford a significant degree of protection (40% survivors). This is one of the few demonstrations of a therapeutic application of interferon against these particular viruses and more particularly in a systemic disease situation.

Interferon Inducers

1) Polynucleotides - the spurious nature of interferon induction by single-stranded nucleic (yeast RNA) was again demonstrated with the finding

that only one source of yeast RNA among many gave measurable antiviral protection of cells *in vitro*. In general single-stranded nucleic acids must be complexed with a polybasic substance such as neomycin to be inducers of interferon. Additionally, in the only active yeast RNA preparation, contamination with a small amount of double-stranded material could not be ruled out[14]. This lends further support to the argument that only double-stranded polynucleotides or suitably complexed single-stranded polynucleotides can induce interferon exogenously. One series of experiments, however, holds that for interferon produced at least by Newcastle disease virus (NDV) the input single-stranded viral RNA is the stimulus for interferon production[15]. The first example of a naturally-occurring double-stranded RNA isolated as a contaminant from a non-*Penicillium* species is that from *Aspergillus foetidus*, and this has demonstrated protective activity in mice against lethal EMC or SFV infections[16]. Recent experiments with T_4 phage indicate that it will induce interferon both *in vitro* and *in vivo*. It was shown that neither of the components - the particulate coat (T_4 ghosts) nor the free double-stranded DNA were active inducers; and it was therefore concluded that the intact phage particle allows the encapsulated DNA to reach the site of action, whereas the free DNA would be expected to have been degraded[17]. This constitutes one demonstration of a double-stranded DNA as an interferon inducer. Interferon induction by double-stranded RNA was shown to be a function of nucleotide chain length; polycations were shown to enhance uptake of these materials[18]. A 2 hour preincubation of nucleotide duplexes at 37° markedly increases their *in vitro* antiviral activity, but has a much less marked but real effect on interferon production *in vitro* or *in vivo*[19]. The bulk of the work with nucleotide duplexes has been carried out with poly IC. From a study in Vero cells (a monkey kidney cell line) which are unable to produce interferon but can be protected by exogenous interferon, it was concluded that since poly IC did not protect this cell type then interferon must be the sole mediator of the poly IC antiviral effect[20]. It is thought that induction of interferon by poly IC is a metabolic process requiring protein synthesis and is not simply a release of stored, preformed interferon[21]. From studies of the refractory state seen after induction of interferon by poly IC it was concluded that a feedback mechanism on interferon synthesis may be operative[22].

The spectrum of *in vivo* protective activity by poly IC continued to be broadened this year. Interestingly, it was shown that poly IC can inhibit the growth of tobacco mosaic virus in plants[23]. It was also shown that the protective effect in mice against vesicular stomatitis virus (VSV) was due to interferon[24]. Injection of 1 mg/kg of poly IC either 24 hours before or after infection with lethal rabies virus (25 LD_{50}) protected essentially all rabbits. High titres of neutralizing serum antibody were found in all survivors and further, all survivors were resistant to reinfection by rabies virus[25]. A significant protective effect of intraperitoneally administered poly IC was demonstrated against both intracerebrally administered herpes simplex virus (HSV) and EMC. Although the dose of EMC virus did not alter the results substantially, the effect against HSV was remarkably sensitive to dose, such that good protection was only seen against 1 $TCID_{50}$ of HSV[26].

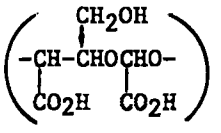
Poly IC is also thought to influence immune phenomena[27] and inasmuch as there is probably a close tie between recovery from virus infection and the immunological system of the host, it is considered of interest to briefly mention some effects observed with poly IC. Because of an enhancement of interferon production with poly IC in mice pretreated with Freund's complete adjuvant and an increase in the number of interferon-forming cells in the spleen, it was concluded[28] that interferon production, immune response and phagocytosis were closely related phenomena. It was also shown that poly IC potentiated the vaccination response to Japanese B encephalitis virus, giving a 4-fold enhancement of neutralizing antibody in serum[29]. A similar but time-dependent adjuvant effect of poly IC was seen in that fewer spleen cells from poly IC treated mice were required to produce a graft vs host reaction[30]. Intravenous poly IC failed to inhibit the growth of trachoma agent in the rabbit eye, but markedly suppressed the ocular lesions produced by this agent. This prompted the authors to speculate that poly IC was enhancing the immunological responsiveness of the host, as it caused a decreased inflammatory response to trachoma agent[31]. In additional studies where poly IC provides protection to mice against a variety of Gram-positive and negative bacterial infections, this is due presumably not to interferon but to a heightened host immune response[32].

Further toxicological studies with poly IC have shown it to be toxic to the vascular and hepatic systems of mice, dogs and rats,[33] and on the endothelial cells of the small blood vessels of the chick cerebellum[34]. The diffuse severe necrosis of villous epithelium in the rat seen after poly IC administration is enhanced some 1000-fold in adrenalectomized rats[35]. There is some conflict with regard to toxicity in the rabbit eye, however, because in one study 1 mg. of poly IC given intravenously produced lens opacity in the rabbit eye which persisted for over a month[36]; in a second study with infected rabbits it was shown that a 1.3 mg. intravenous dose of poly IC exhibited a positive effect on the recovery from a herpes ocular infection but no lens opacity was noted[37].

Some early studies of poly IC in man have shown that a low titre of interferon (1:16) is achieved after intravenous administration[38]. No effect on prolongation of life or alleviation of the disease conditions was seen in terminal cancer patients after intravenous administration of poly IC but the only side effect noted was mild fever[39]. Poly IC was also administered as a nose drop formulation to volunteers challenged with rhinovirus 13 and influenza A_2/Hong Kong/68. In the rhinovirus 13 challenge experiment poly IC was considered effective as there was a lessening of cold symptoms and a markedly decreased shedding of virus. The results with Hong Kong influenza were not as significant[40]. In considering any human trials, especially when poly IC is given by the intravenous route, it is well to keep in mind the finding that human serum rapidly degrades poly IC[41].

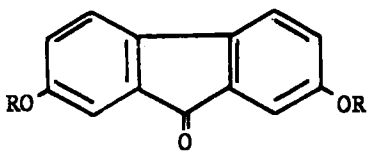
2) Other Synthetic Polymers - There was very little activity in this particular area during the past year. The most significant finding were the reports[42,43] on polyacetal carboxylic acids. These materials are

obtained by sequential oxidation of amylose by periodate and chlorite respectively, and are considered to contain relatively large amounts of the repeating unit 1. The structure-activity relationships are similar to those found for other polycarboxylic acids i.e. the activity is a function of molecular weight and requires the presence of a high density of negative charge. The materials induce small amounts of circulating interferon after intraperitoneal administration and protect mice against mengo, vaccinia, SFV, and influenza APR-8 viruses. The single major advantage over polyacrylic acid type polymers is the apparently lower toxicity, which may be a reflection of the more bio-degradable nature of the backbone.



1

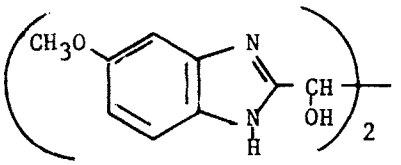
3) Tilorone - Perhaps the greatest excitement of the year was the disclosure of the interferon inducing diamine Tilorone (2)[44-48]. This material induced an antiviral state after oral administration to mice against a range of virus infections which was dose-dependent and thought to be due to circulating interferon[44,47]. Maximum interferon titres were achieved within 24 hours systemically and 48 hours intracranially after oral dosing, and maximum protection was provided when virus was administered at one of these times depending on the route of infection[45]. Tilorone exhibits good oral protection against SFV at 250 mg/kg when given up to 72 hours prior to virus challenge, is optimum at 24 hours prior but does exhibit significant protection at 3 hours after challenge[49]. A hyporeactive state is produced after Tilorone administration[49]. In another study a direct relationship between the dose of Tilorone, serum interferon titres and the survival of mice infected with VSV also prompted the conclusion that Tilorone exerts its antiviral effect via interferon[50]. These same authors[50] also established an oral therapeutic index of 15 and an intraperitoneal therapeutic index of 2.5 for treatment of this infection. An examination of mouse tissues 24 hours after dosing with Tilorone demonstrated that interferon titres are highest in lymph nodes and thymus[50]. Tilorone was chosen as the optimum member of the fluorene series[51]. An examination of other aromatic nuclei indicated that activity could not be predicted as some compounds with grossly similar structural features were essentially inactive whereas others approached the activity of Tilorone. Seemingly minor structural modifications even within the fluorene nucleus caused major changes in activity. Inasmuch as all the possible structural permutations were not presented, it would be inappropriate to attempt to summarize structure-activity relationships here. Toxicological studies show that Tilorone causes changes in the hematopoietic and reticuloendothelial systems of the mouse, rat, dog and monkey, which regress on cessation of drug treatment[52]. Although the significance of these observations is not clear, these authors[52] also found similar results with poly IC. Data concerning the efficacy and safety of Tilorone in man will obviously be awaited with great interest.



R = $CH_2CH_2N(CH_2CH_3)_2$

2

Benzimidazoles - Although antiviral activity was described for certain benzimidazole derivatives[53,54] perhaps the most striking was found in a series of papers concerning bis-benzimidazoles[55-58]. Structural modification of a series of bis-benzimidazoles having features similar to 3, where changes were made in the position and nature of aromatic substituents, in the length and substitution pattern of the connecting carbon chain, and in the substituents at N_1, demonstrated that there were remarkably few structural variants that retained antiviral activity. Optimal for activity were a 5-methoxyl or ethoxyl moiety, a 2-carbon connecting chain which is unsubstituted or substituted by hydroxyl (or acetoxyl) and no substituent at N_1. The compound chosen for further study was (S,S)-1,2-bis(5-methoxy-2-benzimidazolyl)-1,2-ethanediol (3)[55]. This was active *in vitro* both therapeutically and prophylactically against 55 strains of rhinovirus, 3 strains of polio virus and 2 strains of coxsackie virus with *in vitro* therapeutic indices of 100 or greater[56]. The mechanism of action was reported to be unknown. Pharmacodynamic studies indicated that good serum levels were obtained in mice after oral or intraperitoneal administration and that serum samples contained activity against rhinovirus and other picornaviruses but not against adeno, herpes, myxo and paramyxoviruses, thus establishing the same spectrum of activity *in vivo* as *in vitro*[57]. Further testing in chimpanzees indicated good oral protection at high dose (100 mg/kg/day for 4 days) *vs* 100 $TCID_{50}$ of rhinovirus 30, but severe diarrhea accompanied its use at this level. Testing in chimpanzees at lower dose levels of 15 and 50 mg/kg indicated partial protection *vs* 100 $TCID_{50}$ of rhinoviruses 44 and 49 without side effects[58]. Further toxicological studies in primates,[58] dogs and rats showed that this compound caused severe irritation of the gastrointestinal tract. Activity against polio virus has been described by others for this class of compounds[59].



3

Nucleosides - A review dealing mostly with 5-iodo-2'-deoxy-uridine (IUDR) has appeared[60]. It is claimed[61] that local applications of a 30-40% (w/v) solution of IUDR in 90% dimethylsulfoxide is effective in promoting healing and in preventing local spreading of herpes hominis cutaneous lesions. Several cases describing the use of IUDR in severe life-threatening infections were reported. An infant with severe herpes hominis encephalitis made an apparent recovery after infusion of a total of 400 mg/kg of IUDR[62]. In the treatment of adults with herpes hominis encephalitis, IUDR given as a total of 430 mg/kg infusion was thought to be effective as 67% of a small population (6 patients) survived this grave infection[63]. A total infusion dose of 550 mg/kg of IUDR allowed recovery from herpes simplex encephalitis, but the patient was left with serious sequelae, indicating the need for rapid diagnosis and aggressive treatment[64]. Although no cure was demonstrated with 5-bromo-2'-deoxyuridine (reportedly less toxic systemically than IUDR) in 2 cases of subacute

sclerosing panencephalitis, it was suggested that some improvement was noted in one patient and that there was no further deterioration in a second. It was felt worthy of comment inasmuch as any improvement is worthwhile in this disease state[65].

Although no clinical testing of 5-trifluoromethyl-2'-deoxyuridine, which is reported to be a more potent analog of IUDR, has been carried out, molecular level studies have shown similar modes of action as this moiety is incorporated into vaccinia virus DNA replacing thymidine, and the resulting virions differ in morphology from normal[66].

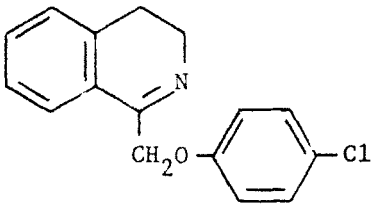
Cytarabine has been used effectively at 100 mg total dose to cure a severe generalized primary herpes simplex infection in an adult[67]. Laboratory studies in vitro with an iodinated derivative (1-β-D-arabino-furanosyl-5-iodocytosine) indicate that this has similar activity to IUDR and in addition is active against IUDR resistant herpes virus[68].

Natural Products - Cylindrochlorin, isolated[69] from the mycelium of a fungus, Cylindrocladium sp., was reported to show activity vs NDV by an agar diffusion plaque-inhibition method[70]. Further structure-activity studies on the epidithiadiketopiperazine acetylaranotin showed that trithia and tetrathia bridged compounds were also in vivo active antivirals, whereas all non-bridged derivatives were inactive[71]. A polypeptide, α-amanitin, was shown[72] to specifically inhibit the replication of influenza virus (fowl plague virus) presumably by inhibition of RNA synthesis although at the concentrations used (25 μg/ml) no effect was seen on host-cell RNA synthesis. Aristolochic acid was shown to exhibit a weak, non-specific protective effect vs Columbia SK virus (75%) when both drug and virus were administered orally[73].

Studies relating to the antiviral properties of rifampicin continued this year. Further studies with vaccinia virus showed that rifampicin prevented the formation of mature progeny presumably by interfering with late transcriptional events, since early events such as DNA replication and assembly of membranes and immature particles were able to proceed in the presence of drug[74]. These effects can be completely reversed by the removal of rifampicin. At this time the irregular membranes previously observed, now undergo transformation to coated envelopes and the return of RNA polymerase activity is coincident with the appearance of DNA containing virus particles[75]. In a study[76] of rifampicin and several analogs on the in vitro inhibition of Shope fibroma virus it was concluded that a hydrazone side-chain was a requirement for activity. A further study proved that the side-chain of rifampicin (1-methyl-4-aminopiperazine) inhibited the replication of vaccinia virus to the same extent and by the same mechanism as the parent drug[77]. The first study claiming to demonstrate in vivo antiviral activity for rifampicin was recently described[78]. Here rifampicin caused as much as a 14-fold decrease in the antibody response to the non-lethal H1 virus infection in hamsters. The same authors however showed it to be inactive against 7 other viruses, offering not much hope for the use of the material in vivo. Finally, a derivative of

rifampicin (the N-demethyl analog) was found to inhibit at relatively high concentrations the now actively studied RNA-dependent DNA polymerase isolated from human patients with acute lymphoblastic leukemia[79].

Isoquinolines - The in vitro antiviral activity of UK-2054 was further broadened with the finding that it it decreased the yield of rhinovirus types 2, 4, 9 and 43 from HeLa cells. Although this drug was previously shown to reduce the incidence of A_2 influenza in man, similar efficacy was not exhibited vs a human rhinovirus 9 infection. This lack of efficacy may well have been related to poor pharmacodynamics with respect to upper respiratory tract tissue, as this compound was not effective against the above-mentioned rhinovirus when tested in a semi-continuous line of human embryo lung fibroblasts[80].



UK-2054

Amines - Amantadine was evaluated both therapeutically and prophylactically, in a double-blind trial in artificially induced A_2 and B influenza in a large study involving 404 subjects. It was 51% effective prophylactically vs A_2 and 73-92% vs the more severe form of A_2. Those subjects who developed influenza had only mild symptoms and a reduced serological response. It was ineffective therapeutically vs influenza A_2. It was also ineffective against B influenza[81]. In a smaller study involving 3 prison populations, amantadine was given therapeutically after symptoms appeared. The authors concluded that it was beneficial as the duration of the febrile response was shortened and other symptoms were less severe, while no differences were noted in virus shedding or antibody titre[82]. In another trial amantadine also exhibited a limited therapeutic effect where patients treated with drug no later than 48 hours after clinical symptoms appeared exhibited a more rapid clearing of some clinical symptoms as well as a decreased shedding of virus[83]. Although at least one report on inhibition of A_2 influenza by other aliphatic amines has appeared[84], perhaps the major clinical interest continues to center around the use of amantadine on Parkinsonism[85,86].

NPT-10381 - Several commentaries and abstracts[87-91] have described, among other reputed properties, the antiviral activity of NPT-10381 which is reportedly the p-acetamidobenzoate salt of the inosine-dimethylaminoisopropanol complex. It is claimed that the material exhibits in vitro and in vivo therapeutic activity against A_1 and A_2 influenza, herpes zoster and vaccinia and that it is active intranasally, orally, or intraperitoneally. It is also reported that human clinical trials in a variety of conditions will begin shortly.

Miscellaneous Synthetics - Several substituted thenoylamides have been reported to be active in mice vs SFV and one against pseudorabies[92].

In vitro activity vs APR-8 virus and vaccinia is reported for two biphenyl derivatives[93]. Ethyl 2-methylthio-4-methyl-5-pyrimidinecarboxylate was active in primary monkey kidney culture against all three types of virulent or attenuated strains of polio virus; no activity has been found against any other virus[94]. A series of 8-hydroxyquinoline derivatives was shown to have some activity vs APR-8 virus in eggs[95]. Two propiophenone derivatives had activity against Ranikhet disease virus in chorioallantoic membrane[96]. A series of 2-substituted phenoxathiins (R=$COCH_3$, $CHOHCH_3$, $CHOAcCH_3$) with in vitro activity vs type III polio virus was described[97]. Several types of compounds, the most active being β-aryl-α-mercaptoacrylic acid, and 1-(o-aminophenyl)-3,4-dihydroisoquinolines have shown activity vs myxoviruses in vitro and also inhibit the enzyme neuraminidase. None showed any appreciable in vivo activity[98]. 2,6,6-Trimethoxy-Δ^3-dihydropyran was active vs influenza type APR-8 in vitro[99]. 3(3,4-Dichlorophenyl)-1-isopropyl-4-methyl-2-imidazolone was active against polio virus in Rhesus monkey kidney cells; however, the compound caused chromosomal abnormalities at twice the dose in rat kangaroo cell culture[100]. Four acetylenic compounds were shown to inhibit the replication of polio virus type I in HeLa cells. They were shown to inhibit the viral RNA polymerase but not the HeLa cell RNA polymerase[101]. Rhodanine (2-thio-4-oxothiazolidine) was found to be highly selective and inhibited only echo virus 12[102]. A series of analogs provided only inactive or slightly active compounds. From a series of hydrazonopyrazol-5-one derivatives 3-methyl-4-phenylamidinohydrazonopyrazol-5-one exhibited pronounced in vitro activity vs pox virus; but no in vivo activity was found[103]. The thiosemicarbazones of 2-formylpyridine, 5-hydroxy-2-formylpyridine, and 5-hydroxy-1-formyl-isoquinoline were found to inhibit HSV, and cytomegalovirus and the enzyme ribonucleotide reductase[104]. The most active triazinoindoles against a large number of rhinovirus strains were 2,2-dimethyl-3-[(5-methyl-5H-as-triazino[5,6-b] indole-3-yl)amino]-1-propanol and 2-methyl-4-[(5-methyl-5H-as-triazino [5,6-b] indole-3-yl)amino]-2-butanol[105]. The dithiosemicarbazone of 2-oximino-1,3-indanedione is active in vitro vs vaccinia[106]. Several systems closely related to isatin-β-thiosemicarbazone, viz benzo[b]thiophene-2,3-diones, 4-oxotetrahydro-4,5,6,7-benzo[b]thiophenes and especially 1,2-benzoisothiazol-3-carbohydrazide have good in vitro activity vs polio virus[107].

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Chapter 14. Antifungal Agents

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Reviews - A thorough review of the clinical use of nystatin discusses all available formulations and all indications.[1] The use of griseofulvin is similarly reviewed.[2] An invaluable review of the treatment of the systemic mycoses gives current data on the mechanisms of antifungal chemotherapy, as well as a summation of the clinical experience with griseofulvin, saramycetin, 5-fluorocytosine, clotrimazole (Bay b5097), and the polyene antibiotics.[3] The three leading antifungal antibiotics, nystatin, amphotericin B, and griseofulvin are also reviewed in a new text.[4]

Methods - The detection and preclinical evaluation of new antifungal agents is facilitated by a new test procedure. Single doses of potential agents are administered to infected mice previously X-irradiated. The procedure gives a more uniform "take" of the various infections, and reduces the time of the test procedure.[5]

Since the therapeutic outcome in the treatment of systemic mycoses with amphotericin B is related to the serum level achieved, careful monitoring is desirable. A bioassay has been proposed that gives precise and accurate results. Low levels of the drug were detected in the serum up to 7 weeks after cessation of therapy.[6]

Clinical Experience - Amphotericin B administered intravenously continues to be a mainstay in the treatment of the systemic mycoses. As experience has been built up, a number of reviews of the action of the drug in various diseases have been published. In chronic pulmonary histoplasmosis, a review of the treatment of 408 patients is presented. In spite of the fact that low doses eradicated the organism in the sputum, a dosage of 35 mg/kg of body weight was required to decrease the case fatality ratio.[7] In the progressive disseminated form of the disease a dosage of 25 mg/kg is life-saving but higher dosages are preferred.[8] In extracutaneous sporotrichosis, several papers indicate the superiority of amphotericin B over iodides or hydroxystilbamidine isoethionate.[9,10] In a review of 109 patients with chronic pulmonary coccidioidomycosis, it was observed that all patients receiving more than 30 mg/kg of amphotericin B did well, whereas those receiving less did poorly. Less drug was required than was the case with the disseminated form of the disease.[11] With systemic North American blastomycosis, 14 of 16 cases achieved partial or complete remission with total dosages ranging from 0.39 to 60 g of drug.[12] The case for intravenous amphotericin B in pulmonary aspergillosis is not as clear, although, there is an impression that it is of some value.[13,14] It appears ineffective when patients also have aspergillomas. An interesting procedure is the instillation of a paste containing amphotericin B or nystatin by repeated intracavitary needling for the treatment of patients with aspergillomas. Results are reported as excellent.[15] It appears that amphotericin B administered by the intravenous route may not reach the aspergilloma in a therapeutic concentration. Amphotericin B proved effective in disseminated cryptococcosis that followed kidney transplantation, with its concomitant treatment with antibacterial antibiotics and immunosuppressive agents.[16] The drug was also effective in several cases of ocular cryptococcosis.[17] Administered orally at a dose of 1.5 to 2 g per day for 9 to 12 days, amphotericin B eliminated *Candida albicans* from the stools of 21 of 22 patients.[18]

The tetraene antifungal antibiotic, pimaricin, has been shown to be effective when applied topically in experimental *C. albicans* oculomycosis in rabbits.[19] In human medicine, corneal ulcers caused by *Fusarium* and *Cephalosporium* sp. have been treated successfully with topically applied pimaricin ointment.[20,21] A short review suggests that pimaricin is no more effective than nystatin in the treatment of candidal vaginitis.[22]

The production of levorin, a heptaene antifungal antibiotic, is being studied vigorously in the U.S.S.R.[23,24] Toxicity studies in rats showed that there was a strong teratogenic effect when the drug was administered orally in repeated doses of 100 mg/kg. Deaths occurred with single doses of 500 mg/kg or more.[25] In the clinic, the drug was administered orally in the treatment of various *C. albicans* infections of visceral organs. Improvement was reported in cases of candidal pneumonia, cholecystitis, and colitis. Levorin was reported as superior to nystatin in these disorders,[26] although the rationale for employing nystatin, an agent that is not absorbed from the gastrointestinal tract, to treat pneumonia and cholecystitis is not clear.

Analogs of griseofulvin prepared by modification of the 5′ position proved to be less active than the parent compound *in vitro* and *in vivo*.[27,28] In 3 of 14 patients treated with oral griseofulvin, there was a rise in erythrocyte protoporphyrin that may have been secondary to hepatic toxicity.[29] Griseofulvin has been reported as effective in the treatment of Raynaud's disease, a vasospastic disorder.[30]

First clinical studies with 5-fluorocytosine have been reported. Doses of 100 mg/kg per day for as long as 87 days were administered orally to ten patients with severe candidosis. Cures or a favorable response were achieved in more than half the cases. No toxic symptoms were observed.[31] Sixteen patients with cryptococcal meningitis were treated with 4 to 6 g per day for 30 to 111 days. Cures were achieved in half the cases. Toxic symptoms were not serious.[32] The drug proved ineffective in experimental coccidioidomycosis in mice.[33]

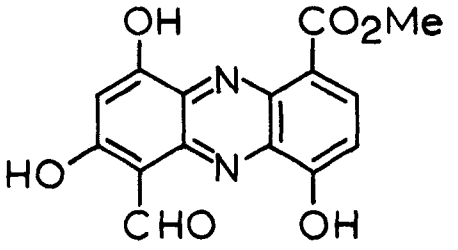
Clotrimazole (Bay b5097) will soon be on clinical trial in the U.S.A.[34] Clinical studies in Germany demonstrated the compound to be active by the oral route in single cases of candidal pneumonia and bronchitis, and aspergilloma,[35,36] but not in a case of chromomycosis.[37] High doses produced liver hypertrophy in rats. As an inducer of liver oxidative enzymes, clotrimazole is as active as phenobarbital.[38]

Miconazole nitrate, 1-[2,4-dichloro-β-(2,4-dichlorobenzyloxy)phenethyl]imidazole, is reported effective both orally and topically in experimental infections of guinea pigs caused by *Trichophyton mentagrophytes*, *T. canis*, and *C. albicans*. In humans it was very effective in the topical treatment of tinea pedis caused by a variety of *Trichophyton* sp.[39]

Haloprogin (M-1028), 2,4,5-trichlorophenyl-γ-iodopropargyl ether, was active when applied topically in experimental dermatophytic infections of guinea pigs. It appeared to be approximately as active as tolnaftate.[40]

In a review of thiabendazole, 2-(4′-thiazolyl)benzimidazole, a drug used as an anthelminthic, its antifungal properties were discussed. Its usefulness for the treatment of the superficial mycotic infections is being investigated.[41] Study of the mode of action of thiabendazole suggested that the primary site of action is inhibition of the terminal electron transport system of the mitochondria.[42]

New Antifungal Agents - The structure of lomofungin, 1, a new phenazine antibiotic, has been determined by degradation studies.[43] This antibiotic is active against Gram-positive and Gram-negative bacteria and fungi.



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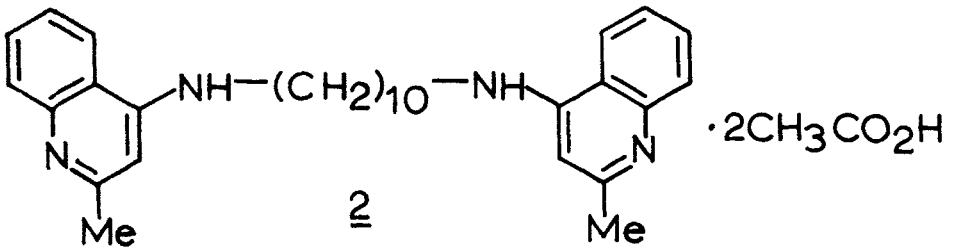
A detailed account of the determination of the structure of LL-Z1271α has been published.[44] The structure and biological properties were summarized in last year's Annual Reports in Medicinal Chemistry (pp. 132-3). A peptide antibiotic, having good activity *in vitro* against *C. albicans*, is produced by a strain of *Aspergillus rugulosus*.[45] Acid hydrolysis indicates that aspartic acid, glutamic acid, glycine, alanine, threonine, valine and isoleucine are components. The production, isolation, and chemical and biological properties of fumigachlorin, an antibiotic produced by a fungus, have been studied.[46] Fumigachlorin has a molecular formula of $C_{16}H_{25}Cl_2NO_4$, and has strong activity *in vitro* against some filamentous fungi. An antibiotic, inomycin, has been isolated from cultures of *Streptomyces griseus var. inomycini*[47] It is active *in vitro* against *Saccharomyces* species and also has antitumor activity. This antibiotic has the approximate molecular formula, $C_{17}H_{27}N_2O_4$, and appears to be closely related to cycloheximide. Two new antifungal polyene antibiotics have been reported; genimycin, a pentaene produced by an *Actinosporangium* species[48] and tbilimycin, a heptaene.[49]

Several derivatives of pyrrolnitrin have been obtained by the metabolism of substituted tryptophans by *Pseudomonas aureofaciens*. For example, tryptophan, substituted at the 5-,6- or 7-position with fluorine, or at the 5- or 7-position with methyl, gave fluorinated and methylated pyrrolnitrins, respectively.[50] Several 3-phenylpyrroles related to pyrrolnitrin were synthesized.[51] Some derivatives that lacked a nitro group on the phenyl ring had stronger antifungal activity and a broader spectrum of activity *in vitro* than did pyrrolnitrin. A new synthetic antifungal agent, 4,4'-(decamethylenediimino)diquinaldine acetate salt (1:2) (2), has activity *in vitro* against *Staphylococcus aureus, Streptococcus haemolyticus, C. albicans, T. mentagrophytes* and *Trichomonas vaginalis*. It was effective in the treatment of skin and mucosal infections due to these organisms.[52] Anticandidal properties of derivatives of β-nitrostyrene have been investigated.[53] Of the derivatives examined, 4-bromo-β-methyl-β-nitrostyrene had the best activity. This compound was effective against visceral candidiasis in mice and in the treatment of candidal lesions on rabbit skin.

Chemical and Physical Studies of Antifungal Agents - Structural studies of nystatin[54] have indicated that the attachment of mycosamine is at C-19. These studies also established the pyranose nature of the mycosamine ring and proved the lactone closure to C-37. Except for



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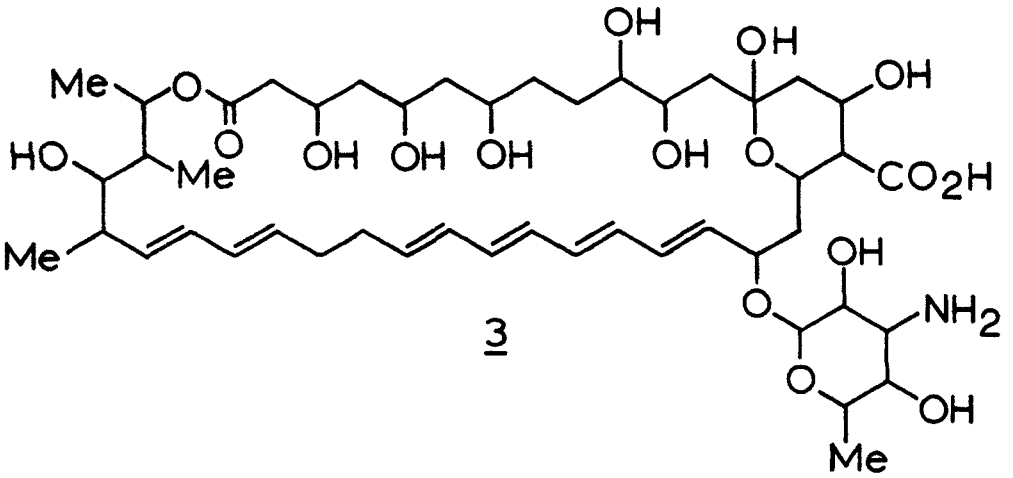
the stereochemistry, the structure, 3, of nystatin has been completed by this work. The



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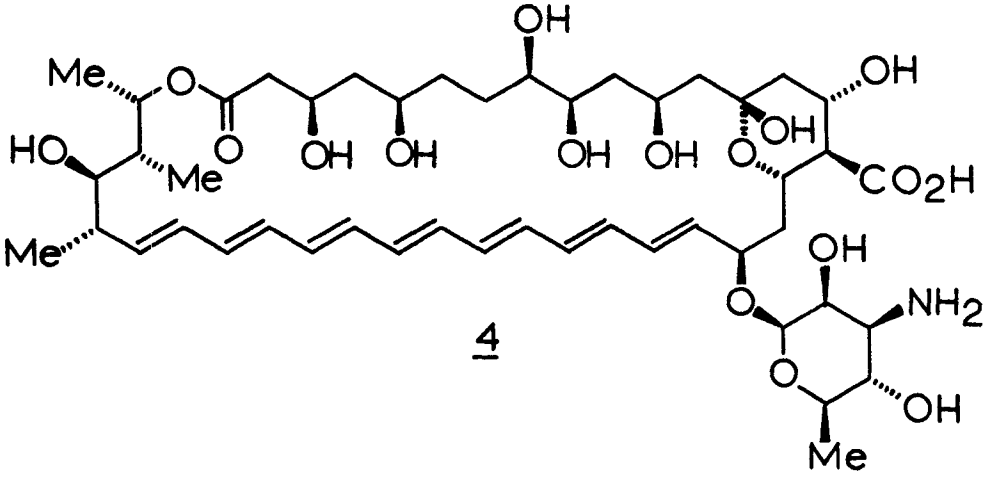
total structure of amphotericin B has been established as 4 by chemical studies[55] and by X-ray crystallography of the *N*-iodoacetyl derivative.[56] Chemical studies on flavofungin[57] have led to the conclusion that this antibiotic is a mixture of two pentaene macrolides that have the same structures as the components of mycoticin, except possibly, for the configuration of one or more asymmetric centers. The biosynthesis of mycoticin has been studied, using labelled precursors.[58]

The structure of X-537A (5), an antibiotic related to nigericin and monensin, has been determined by X-ray crystallography.[59,60] The absolute configuration is based on the Cotton effect exhibited by a degradation product.[59] The structure of nigericin (polyetherin A) (6, R=OH) has been determined by chemical degradation and spectroscopy[61] and by X-ray crystallography of the silver salt.[62] The absolute configuration was determined by anomalous dispersion. The structure of grisorixin, a new antibiotic,[63] has been determined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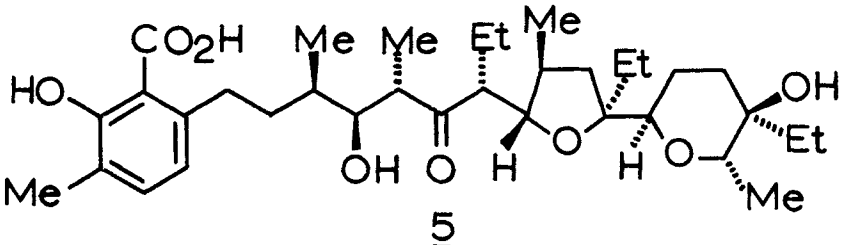
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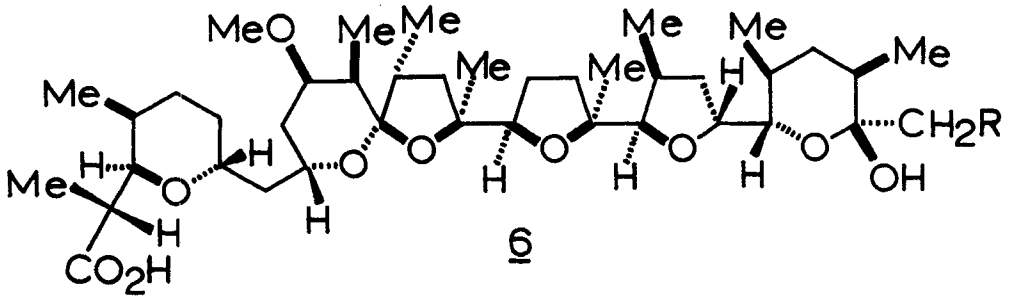
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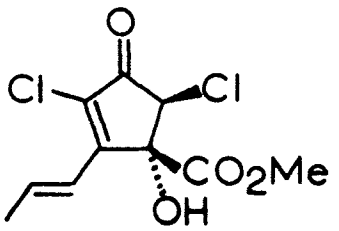
by X-ray crystallography of the silver salt.[64] The structure reported[64] is the enantiomer of 6, R = H.

The total synthesis of racemic cryptosporiopsin, 7, has been reported.[65] This material is half as active as the naturally occuring dextrorotatory enantiomer.



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Biological Studies of Antifungal Agents - The mode of action of pyrrolnitrin has been studied by use of monkey kidney cells, rat liver mitochondria and beef heart submitochondrial particles.[66] These experiments indicated that pyrrolnitrin and "reduced pyrrolnitrin" (having an amino group in place of the nitro group) inhibit respiration of mitochondria, probably by blocking electron transfer between dehydrogenases and cytochrome components of the respiratory chain.

The basis for the nephrotoxicity of amphotericin B has been studied, using turtle bladder as a model system. In one study,[67] exposure of turtle urinary bladder to amphotericin B resulted in changes in the electrophysiological properties of the bladder and in morphological changes in the mucosal cells. The results indicate that the primary effect is on luminal plasma membranes. In another study,[68] the impairment of urinary acidification was investigated, using turtle bladder that had been exposed to amphotericin B. The results indicate that this defect is attributable to increased passive permeability of the luminal membrane and not to failure of active transport.

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Section IV - Metabolic Diseases and Endocrine Function

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Chapter 15. Prostaglandins and Related Compounds

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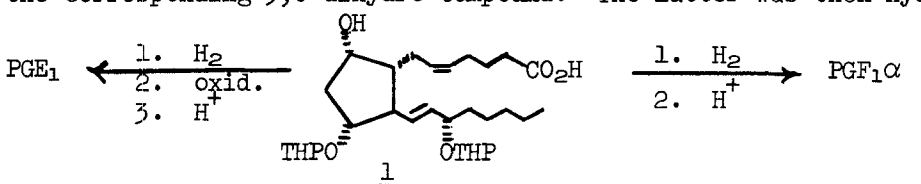
The fact that nearly three-quarters of the world's technical literature on prostaglandins, amounting to over one thousand publications, has appeared in the last three years is evidence of the rapidity with which the area of prostaglandin research is growing. At least fifteen major pharmaceutical firms and numerous academic laboratories maintain active research efforts in this field. It is likely, however, that even the extensive work reported in the literature thus far may still represent only a preview of things to come. With the recent development of several synthetic routes to prostaglandins which should be amenable to large scale operations, progress in clinical and biological areas will be even more rapid than before.

This report will summarize advances made during the past year in chemical, biological and clinical prostaglandin research. Emphasis will be placed upon the chemical area, since numerous more extensive reviews have appeared recently on the other aspects.

I. Syntheses of natural prostaglandins - E.J. Corey and his associates have recently disclosed several modifications of their already efficient 16-step synthetic route reported and reviewed earlier.[1,2] The bis-tetrahydropyranyl ether 1, the precursor of PGE_2 and $PGF_2\alpha$,[1] could be hydrogenated in methanol at -20° with a 5% palladium on carbon catalyst yielding the corresponding 5,6-dihydro compound. The latter was then hydro-



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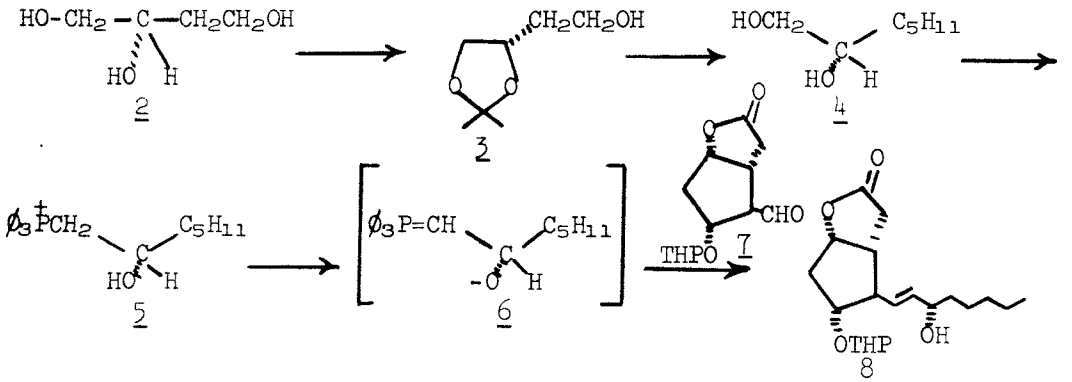
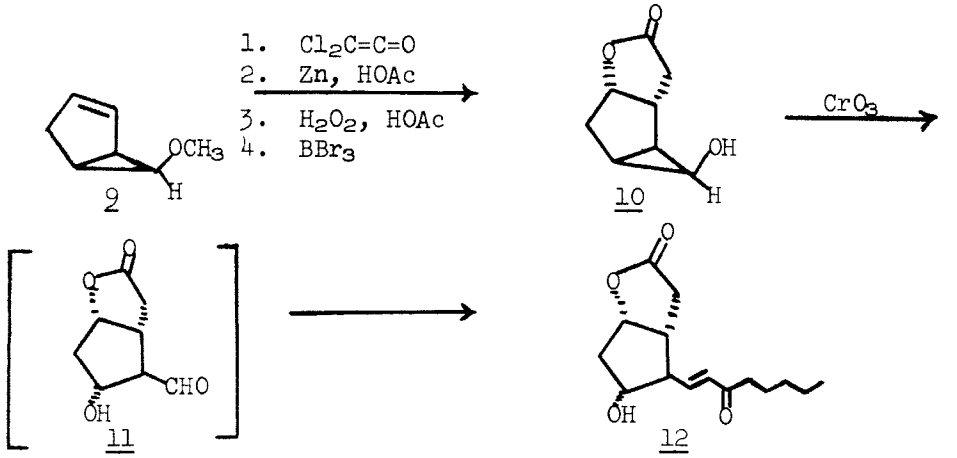
lyzed to $PGF_1\alpha$ (80% yield) or oxidized and then hydrolyzed, affording PGE_1 in 64% yield.[3] These transformations constitute the first example of the synthesis of the four primary prostaglandins from a single precursor. This reduction procedure has been used commercially (New England Nuclear) to prepare tritium-labeled PGE_1 and $PGF_1\alpha$ of high specific activity. Koch and Dalenberg[4] have found that homogeneous hydrogenation of PGE_2 itself with $RhCl(PPh_3)_3$ catalyst afforded PGE_1 in yields up to 50% and have made tritiated PGE_1 by this method.

Corey has further modified his earlier route by developing a method for the introduction of the C-12 side chain containing the required 13,14-trans double bond and the 15(S)-configuration.[5] L(-) malic acid was reduced to (S)-1,2,4-butanetriol (2) and the latter was chain extended by three carbons via the acetonide 3. The triphenylphosphonium salt 5 de-



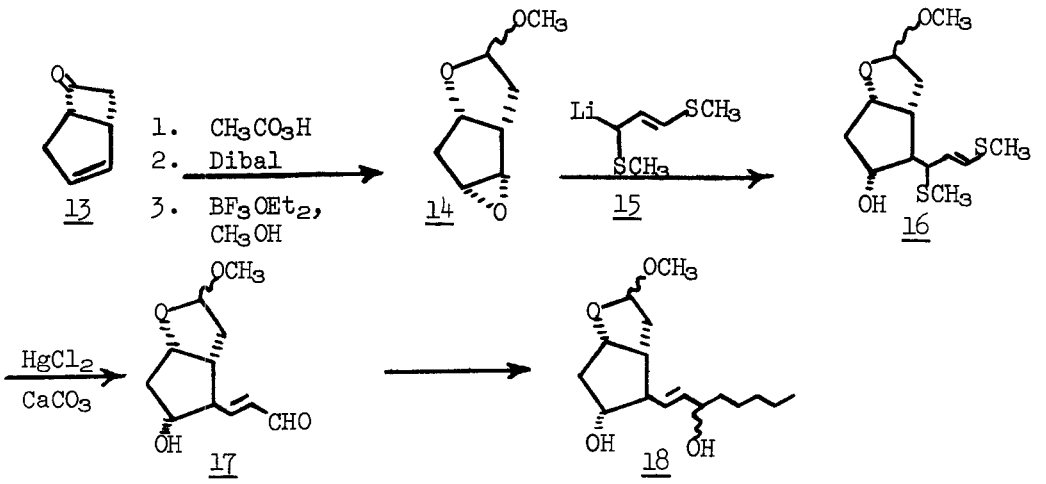
rived from 4 was converted to the β-oxido phosphonium ylid[6] 6 which reacted with the appropriate tetrahydropyranyl lactone aldehyde[1] 7 to afford stereospecifically the required unsaturated lactone 8 in better than 50% yield. Conversion of 8 to PGE_2 and $PGF_2\alpha$ was accomplished as described earlier.[1] With the incorporation of the modification just described, Corey's synthetic route to prostaglandins is now completely stereospecific.

Several alternative routes to key intermediates were investigated by Corey and his group. In one approach,[7] the key step was the position-spe-



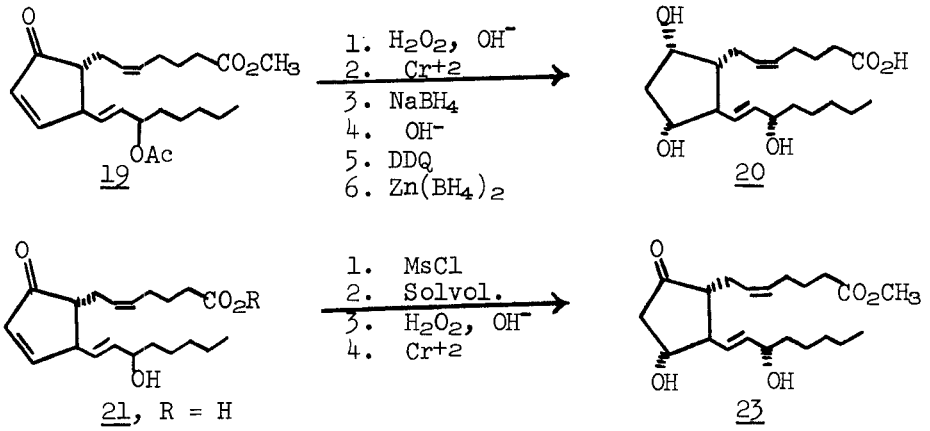
cific and stereospecific addition of the elements of dichloroketene to the bicyclic ether 9. Subsequent dechlorination of the α-dichloroketone, Baeyer-Villiger lactonization, and ether cleavage gave the endo cyclopropanol 10 in 44% yield from 9. Chromic acid oxidation of 10 produced, along with two other products, hydroxyaldehyde 11 which was not isolated but was treated immediately with the sodio derivative of 2-oxoheptylphosphonate. The Wittig product 12, closely related to an intermediate from Corey's earlier route,[1] was isolated in 12% yield. It is the low efficiency and selectivity of the transformation 10 ⟶ 11 that makes this approach inferior to the one presented earlier.[1]

A second approach[8] involves conversion of the unsaturated bicyclobutanone 13 to the oxido acetal 14 and opening of the epoxide with 1,3-bis



(methylthio)allyllithium 15, a nucleophilic equivalent of the -CH=CH-CHO group. Hydrolysis to yield 17 (30% from 14), followed by addition of n-amyllithium gave a mixture of alcohols 18 which offered a tie-in point with Corey's earlier synthesis.[1] The route from 13 to $PGF_2\alpha$ is attractive by virtue of its directness but the low specificity exhibited in the epoxide opening step is a decided disadvantage.

A research group from The Upjohn Company has recently disclosed the conversion of prostaglandins derived from the marine organism Plexaura homomalla to $PGF_2\alpha$ and PGE_2 methyl ester.[9] Conversion of 15(R)-PGA_2 acetate methyl ester 19, isolated from Plexaura homomalla in 1.3% yield,[10]

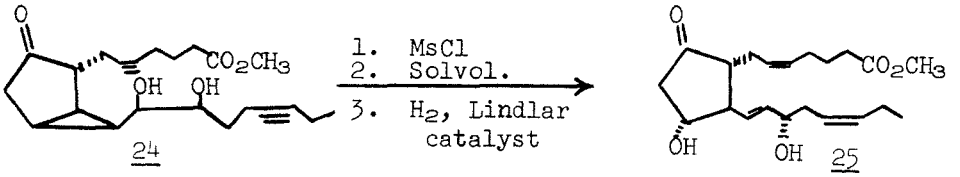


to 15(R)-PGE_2-diester was accomplished by epoxidation and reduction of the α,β-epoxyketone mixture formed. After separation of the C-11 epimeric mixture by silica chromatography ($11\alpha/11\beta$ = 75/25), the 11α-isomer was reduced to the PGF series where separation of C-9 epimers ($9\alpha/9\beta$ = 68/32) again required chromatography. Hydrolysis in base, selective allylic oxidation[11] with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), reduction of the C-15 ketone[1] and separation of C-15 alcohol epimers [15(S)/15(R) =

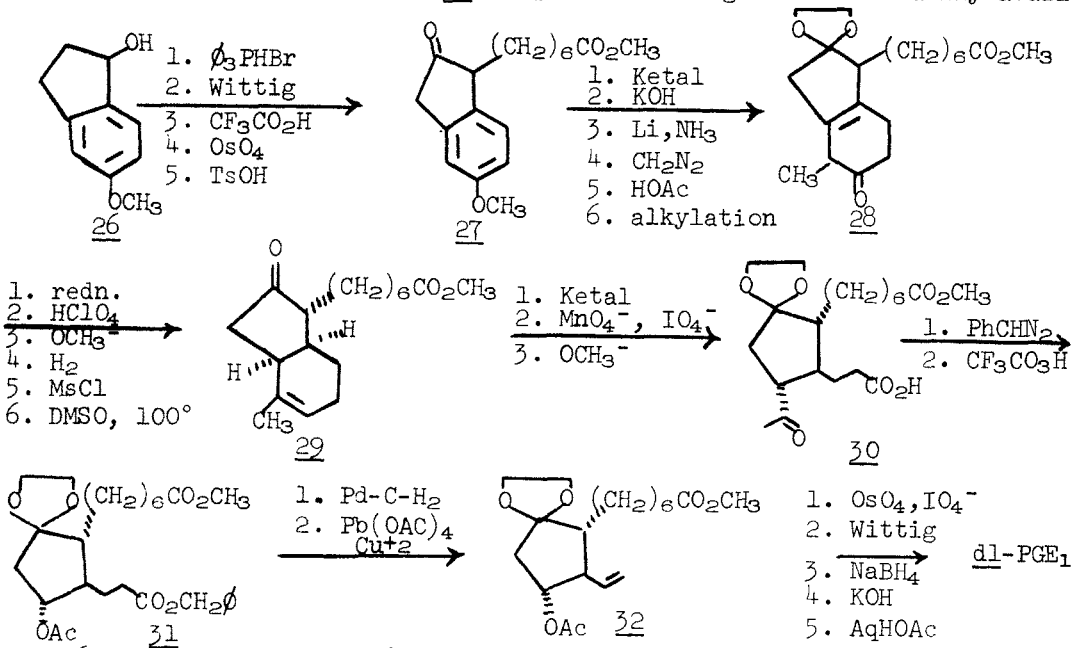
73/27)] completed the synthesis of $PGF_2\alpha$. Advantages of this partial synthetic route are that few chemical steps are involved and, in contrast to total synthesis, no resolution is required. A disadvantage is the generation of epimeric mixtures at C-9, C-11 and C-15, all requiring chromatographic separation.

A modification of the above route led to PGE_2, methyl ester 23. Solvolysis of a C-15 methanesulfonate derived from 15(R)-PGA_2 methyl ester (22) [15(R)-PGA_2, 21, is a minor product from Plexaura homomalla] gave among other products 15(S)-PGA_2 methyl ester. Epoxidation and reduction as before afforded PGE_2 methyl ester 23 after chromatographic separation of C-11 epimers. These partial syntheses represent the first reported conversion of non-mammalian natural products into primary prostaglandins.

PGE_3[12] had until recently been available only from natural sources and in very limited amounts. Upjohn chemists[13] have described the synthesis of dl-PGE_3 methyl ester 25 via endo-bicyclohexane intermediates (e.g. 24), an approach which has been discussed in detail earlier.[14]



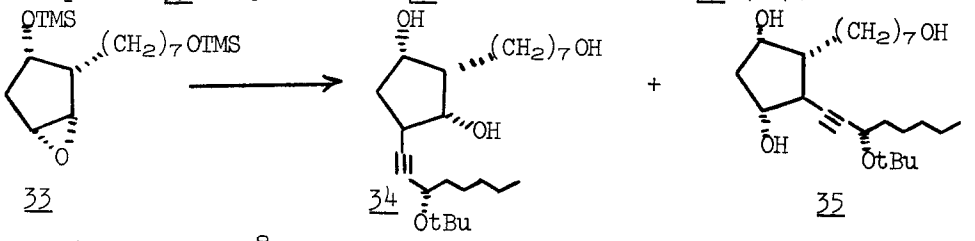
A new approach to prostaglandin synthesis has been used by Merck chemists for the synthesis of dl-PGE_1.[15] Starting with the readily available 6-methoxy-3-indanol 26, the desired relationship between C-8 and C-12 (prostaglandin numbering) was achieved through construction of a cis-hydrindanone system in which an exo-carboxyl side chain arrangement is ther-



modynamically favored (29). The trans stereochemistry between C-11 and C-12 was then generated by equilibration of a C-11 acetyl group, yielding 30. The fact that no yields are reported makes a comparative evaluation of this route difficult; however, the major disadvantage of this general approach appears to be its length (29 steps).

II. Syntheses of structurally modified prostaglandins - The synthesis of dl-7-oxa-PGE_1 was reported by J. Fried and his coworkers[16] and followed an approach similar to that used earlier to make other 7-oxa analogs.[17] A key reaction in this sequence was the opening of an epoxide with diethyl octyl alane,[18] which served to introduce the alkyl side chain. A mixture of dl-7-oxa-PGE_1 and the corresponding 15-epimer exhibited 4 x 10^{-4} the activity of PGE_1 in a gerbil colon smooth muscle assay.[16]

Fried's group has also described the synthesis of optically active 7-oxa-$PGF_1\alpha$[19] and $PGF_1\alpha$ alcohol.[19] Conversion of the latter, prepared using key reactions from the 7-oxa syntheses, into natural $PGF_1\alpha$ is anticipated. The only real problem in this synthesis is the unfavorable opening of epoxide 33 to yield more 34 than the desired 35 (20%).



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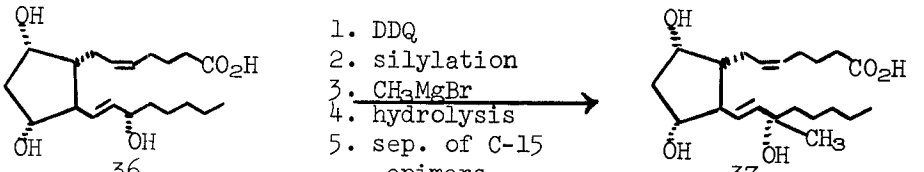
Upjohn chemists[9] have disclosed the synthesis of the 15-methyl analogs of $PGF_1\alpha$, $PGF_1\beta$, $PGF_2\alpha$ and $PGF_2\beta$ using the scheme indicated below for the $PGF_2\alpha$ case (36 → 37). 15-Methyl-PGE_1 and-PGE_2 were prepared via oxidation of the corresponding 15-methyl-$PGF_1\beta$ and -$PGF_2\beta$. These are among the first analogs to be reported which are not substrates[20] for the



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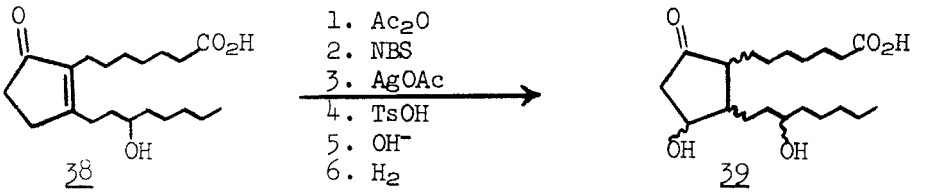
prostaglandin 15-dehydrogenase[21,22] (responsible for the initial deactivation of prostaglandins in man.[22]) Nonetheless, they retain prostaglandin-like activity.

Earlier, N.A. Nelson[23] reported the preparation of several dl-3-oxa prostaglandin analogs, synthesized via bicyclohexane intermediates.[14] These analogs were designed to block β-oxidation of the prostaglandin carboxyl side chain.[24,25]

Two different syntheses have been described for isomeric mixtures containing dl-13,14-dihydro-PGE_1.[26] Klok, Pabon and van Dorp (Unilever)[27] converted intermediate 38 in six steps to a mixture 39 containing isomers at C-8,11,12 and 15 in low yield. The hydrogenation step resulted in con-



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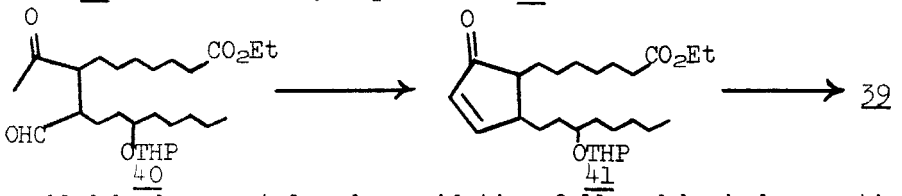
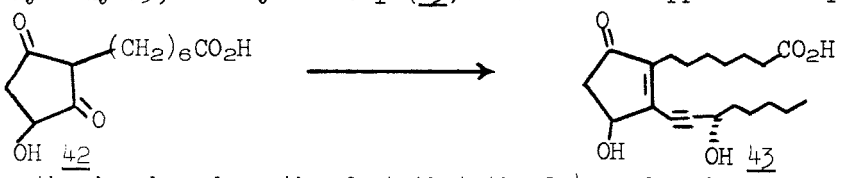
siderable hydrogenolysis of the C-11 hydroxyl group as well. Strike and Smith[28] (Wyeth) utilized an aldol cyclization of a substituted levulinic aldehyde 40 to form the cyclopentenone 41 to which the elements of water



40 41

were added by base-catalyzed epoxidation followed by hydrogenation. As in the previous case a complex mixture of isomers was obtained (mostly trans C_{8-12}). In both cases, the use of hydrogenation as the final step severely limits the versatility of these routes.

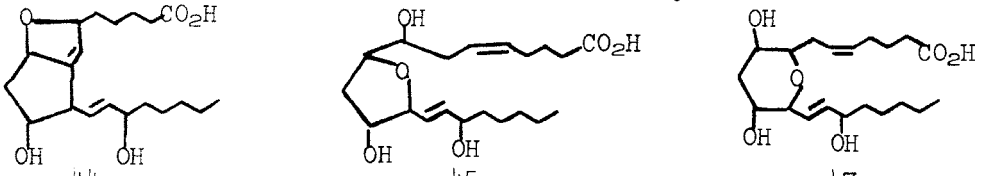
A full paper has appeared by Miyano[29] (Searle) on the synthesis of 15-dehydro-PGB_1, which had appeared earlier in the form of a communication.[2,30] Utilizing resolved 1-octyn-3-ol[31] and a general route disclosed earlier,[32] the Searle group has synthesized optically active PGB_1.[31] Modification of this route, starting from hydroxydione 42, allowed the synthesis of 11-hydroxy-13,14-dehydro-PGB_1 (43). A similar approach to prosta-



42 43

glandin synthesis, based on the fact that the 1,4-carbonyl groups of 1,2,4-cyclopentanetriones can be protected via orthoformic esters, has been investigated by Vandewalle, et al.[33] in a model system.

Pace-Asciak and Wolfe[34,35] found that enzymatic conversion of ara-



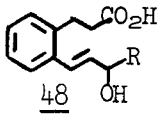
44 45 47

46 5,6-dihydro

chidonic acid into PGE_2 afforded, in addition to PGE_2, compounds 44, 45 and 47. The dihydro derivative 46 was derived from endogenous eicosatrienoic acid. A biosynthetic rationale for the formation of these products was presented.

Finally, Collet and Jacques[36] have synthetized a number of prosta-

glandin "analogs" with the general structure 48.



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III. Biology - A difficulty which has long plagued both biological and clinical prostaglandin research is the lack of availability of reliable, reproducible assay methods for the sub-microgram/gm amounts of prostaglandins in tissues and biological fluids. The methods in common use (enzymatic assay, absorption spectroscopy, fluorescence, glc, bioassay, etc.) have been reviewed by Shaw and Ramwell,[37] but most of these suffer from being either non-specific, too complex, or too inaccurate at the low levels of prostaglandins involved. During the past year, important advances have been made in the development of sensitive, specific assay methods. Jubiz and Frailey[38] have obtained antibodies against PGE_1 and $PGF_2\alpha$ and are currently developing a radioimmunoassay. Levine and VanVunakis[39] reported a radioimmunoassay for PGE_1, A_1 and $F_2\alpha$ based on complement fixation. Jaffe et al.[40] can determine PGA_1 by immunoassay at levels lower than 0.1 picomole (10^{-12} moles) and PGE_1 at less than 0.15 picomole. Burstein et al.[41] recently reported a radioimmunoassay for $PGF_2\alpha$ (picogram range) including the extraction and separation techniques essential for application of the technique to routine analysis. They emphasize the need for a chromatographic separation at least into classes of prostaglandins to avoid cross reactions. Several improved gas chromatographic assays have appeared.[22, 42-44] PGB's and PGF's (as silyl ethers) were determined by van Dorp[42] in nanogram amounts using electron capture detection. Horton et al.[43] reported a combined gas chromatographic-mass spectrometric method for separation, identification and estimation of prostaglandins in amounts down to 10 ng. Samuelsson and Sweeley[22,44] have utilized a novel technique combining gas chromatography, mass spectrometry and reverse isotope dilution (i.e. addition of a deuterated carrier) to analyze for prostaglandin (especially PGE's) at the nanogram level.

In 1970 alone, more than two hundred articles have appeared related to the biology of prostaglandins. An adequate summary of this quantity of material is far beyond the scope of the current review and the reader is therefore referred to numerous other more comprehensive reviews[45-49] which have appeared recently for further information.

The biosynthesis of prostaglandins has been reviewed by Samuelsson[50] and the structural requirements of the substrate fatty acids have been investigated by van Dorp[51] who converted a number of novel unsaturated fatty acids to biologically active prostaglandin analogs (e.g. Δ^2, Δ^3, Δ^4 and Δ^{18}-PGE's). The metabolism of prostaglandins in man, guinea pigs and rats was summarized by Samuelsson.[22] In these species, prostaglandins are degraded by various combinations of four reactions: oxidation of the C-15-hydroxyl, reduction of the $\Delta^{13,14}$ double bond, β-oxidation of the carboxylic acid chain and ω- (and ω-1) oxidation of the alkyl chain. Evidence was presented[22] that in man the above sequence represents the order in which these steps occur. Larsson and Änggård[52] found that prostaglandin 15-dehydrogenase occurs most abundantly in tissues where prostaglandin

biosynthesis is also greatest. This suggests the possibility that in some cases prostaglandins may be generated, utilized and deactivated within single cells.

The relationship between prostaglandins, adenyl cyclase and cyclic AMP is still being unravelled.[49,53,54] In most tissues studied, PGE's mimic the actions of various stimulating hormones. Available evidence suggests that prostaglandins (the E series more than the F's) may serve as intracellular modulators of adenyl cyclase and hence of cyclic AMP levels, although the exact mechanism of this interaction is uncertain. Shaw[55] has pointed out that not all actions of prostaglandins can be accounted for by cyclic AMP formation or inhibition, and also that prostaglandins are not required for the functioning of all adenyl cyclase systems.

An area which may assume future importance is that of prostaglandin antagonists. Eakins, et al.[56,57] reported the antagonism of the smooth muscle stimulating actions of PGE_2 and $PGF_2\alpha$ by polyphloretin phosphate. Although the mechanism of antagonism is not yet clear, it is apparently selective for prostaglandins, as contractions produced by other agonists were not reduced. A dibenzoxazepin hydrazide derivative (SC-19220), prepared by Sanner,[58] was an antagonist of PGE_1 and PGE_2, reported to act by preventing the prostaglandins from reacting with their receptors.

A large effort continues to be expended in more precisely defining the role of prostaglandins in reproductive physiology (Reviews[59-61]). Prostaglandins are present in human semen and menstrual fluid, in the human umbilical cord and amniotic fluid at term and in human blood during labor. Pharriss has hypothesized that $PGF_2\alpha$ may be the long-sought for uterine luteolysin,[62-65] but further work will be required to settle this point. Data has been presented[65] which suggests that the luteolytic mechanism of $PGF_2\alpha$ involves reduction of ovarian blood flow. The luteolytic effect of $PGF_2\alpha$ may be overcome by luteinizing hormone or human chorionic gonadotropin. K.T. Kirton demonstrated that $PGF_2\alpha$ or PGE_2 could be used to interrupt early stages of pregnancy in rhesus monkeys[66,67,68] intravenously, subcutaneously and intravaginally. $PGF_2\alpha$ also lowers plasma progesterone levels within 24-48 hrs of the initial administration.[68] Effects of prostaglandins on the reproductive system of rhesus monkeys are similar to those found in humans[67,68] (e.g. relative potency of PGE_2 vs. $PGF_2\alpha$, resistance to termination of pregnancy during the second trimester of pregnancy, total amounts of PG's necessary to initiate myometrial contractions.) Hence the rhesus monkey is a reasonably good animal model for basic research.

Evidence concerning the role of prostaglandins in the gastrointestinal tract has been summarized recently by Bennett and Fleshler.[69] New concepts relating prostaglandins to various ocular functions have been surveyed by Waitzman.[70] Shio, et al[71] have discussed the effect of prostaglandins on platelet aggregation.

IV. Clinical[72] - Antisecretory: In 1968, Horton[73] found that administration of 10-40 µg/kg of PGE_1 orally produced no inhibition of pentagastrin-

induced gastric secretion in humans. Wilson, et al[74] demonstrated that PGA_1 infused intravenously at 0.5-1.25 μ g/kg/min for thirty minutes decreased both the volume and acidity of histamine induced secretion. PGE_1 was found to significantly inhibit stimulated gastric secretion in man with "tolerable" side effects at a total dose of 4-5 μg/kg administered i.v.[75] (see also page 71, this volume).

Bronchodilation: Human bronchial muscle in vitro is known to be sensitive to PGE's.[76] In preliminary studies PGE_1 (or the less irritating triethanolamine salt) given as an aerosol had no effect on resistance to air flow in normal subjects, but reduced resistance in asthmatic subjects with essentially no effects on ECG, blood pressure or pulse rate.[77]

Nasal patency: Änggård[78] reported that PGE_1 and PGE_2 (10-15 μg) are effective in increasing nasal patency. This increase is the result of constriction of nasal blood vessels. Recent, more extensive studies with PGE_1[79] show that vasoconstriction, when present at all, was fairly long lasting (3-12 hrs) at doses between 37-75 μg (higher doses were irritating).

Hypertension, cardiovascular:[80,81] The most interesting of the prostaglandins for renal and cardiovascular purposes are the PGA's since they possess the cardiovascular and renal effects of PGE's without the smooth muscle stimulating actions of E-series. Also, PGA's exhibit low acute toxicity, are well tolerated by i.v. infusion, and escape rapid deactivation in the pulmonary circulation.[80] Lee[82] and others[83,84] have found the PGA's effective in the treatment of patients with essential hypertension. In fact, the possibility has been suggested that PGA_1 and PGA_2 may normally exert a regulatory antihypertensive endocrine function.[85]

Therapeutic abortion: Therapeutic abortions may be induced, especially during the first trimester of pregnancy, using intravenous infusion of either PGE_1, PGE_2 or $PGF_2\alpha$.[86-90] Bygdeman's group[91] favors the use of $PGF_2\alpha$, while Karim[87,88] prefers the chemically less stable but 8-10 times more potent PGE_2. Vomiting and diarrhea are side effects with either compound. Wiqvist and Bygdeman reported recently[90,92] that administration of $PGF_2\alpha$ directly into the uterine cavity between the fetal membrane and the uterine wall led to abortion with clinical effectiveness comparable to the i.v. route. By the intrauterine route, however, the total dose required is only about 1/10 that necessary i.v. and, thereby, generalized side effects were almost completely eliminated. Karim and Sharma[93] described the induction of abortion in 45 women using intravaginal administration of PGE_2 and $PGF_2\alpha$. Gillespie et al[94] reported three cases of therapeutic abortion using ω-homo-PGE_1. This is the first reported example of the use of a prostaglandin analog as an abortifacient. Bygdeman[90] and Karim[95] have both reported the use of prostaglandins as post-implantation antifertility agents. PGE_2 or $PGF_2\alpha$ intravaginally[95] and $PGF_2\alpha$ intravenously[90] induced menses in women who were 2-7 days past their expected menstrual date.

Induction of labor: Induction of labor at term may be readily ac-

complished by i.v. infusion of either PGE_2 or $PGF_2\alpha$.[96-100] The problem of increased uterine tone encountered on occasion with the PGE's[100] can apparently be overcome using lower doses and longer infusion times.[96,98,99] Intravaginal application of PGE_2 or $PGF_2\alpha$, 2 mg and 5 mg respectively every two hours, has also been used for induction of labor with no adverse side effects noted.[93] Very recently, Karim[101] used PGE_2 and $PGF_2\alpha$ orally to induce labor in 100 patients. The usual doses were 0.5 mg of PGE_2 and 5 mg of $PGF_2\alpha$, repeated every two hours until labor was established. PGE_2 had the higher success rate (79/80). In a double-blind study involving three matched groups of 100 patients each, Karim[95] found PGE_2 better than $PGF_2\alpha$ and the latter better than oxytocin. Two other double-blind trials,[102,103] however, did not find such marked differences between the prostaglandins and oxytocin. Roth-Brandel and Adams[104] also express doubts about the superiority of prostaglandins to oxytocin for labor induction at term. The prostaglandins may find their biggest use in this area for induction of labor before term (when oxytocin is ineffective) and in cases of difficult inducibility.[103] Anderson[103] has emphasized the necessity of using standard definitions of inducibility (e.g. Bishop scores[105]) in all labor induction work and standard definitions of "success" in all abortion studies so that comparison of results from different laboratories can be significant.

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Chapter 16. Atherosclerosis

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Introduction - Atherosclerosis is defined by the WHO[1] "as a variable combination of changes of the intima of arteries consisting of the focal accumulation of lipids, complex carbohydrates, blood and blood products, fibrous tissue and calcium deposits and associated with medial changes." Once formed the advanced plaque seldom regresses, and consequently major research attention has concentrated on prevention of additional deposits or, in the longer view, on the primary prevention of all lesions.

The unfortunate sequelae of atherosclerosis, coronary heart disease (CHD) and cerebral vascular accident are the single largest cause of death in this country. There are over 650,000 deaths per year from CHD and an additional 250,000 per year who succumb to atherothrombotic disease of major arterial vessels in other parts of the body. This total is threefold higher than the fatalities attributed to cancer and accounts for almost one-half the deaths occurring in males aged 45-54. As long ago as 1962, The National Health Examination Survey estimated that 5% of the population between the ages of 18 and 79 had definite or suspected coronary heart disease.

The disease and ensuing lesions have been well documented pathologically and chemically, detailed and accurate descriptions appearing in textbooks as early as 1855.[2] Although literally armies of researchers from diverse fields of specialization have attacked the disease and accumulated mounds of data and much useful information, the pathogenesis of atherosclerosis is still only dimly understood. Further significant developments will probably come from those investigators who can bring to bear training in multidiscipline approaches and are conversant with the more sophisticated techniques constantly being developed.

The American Heart Association Study Group, "Primary Prevention of the Atherosclerotic Diseases," chaired by J. Stamler and A. M. Lilienfeld, have reported[3] on the magnitude of the prevention problem. This report is comprehensive in its background and recommendations and should be read by all who are interested in this aspect of the subject. A broad review of experimental cardiovascular disease has been compiled by Selye.[4]

Etiology - Life style is apparently an important facet in the development of atherosclerosis. Although the disease is universal, its greatest prevalence is generally found among the more technologically developed or affluent countries. This has been underscored in studies with groups of people migrating from areas with low atherosclerotic incidence to regions with high incidence of the disease. Well known examples are Yemenites to Israel, Italians to United States, Irish to Boston and Japanese to Hawaii. Thus, it is not surprising that environmental or risk factors have been investigated as possible contributors to disease development. Diet, lack of exercise, stress, cigarette smoking, hypertension and serum lipid levels have each been correlated with atherosclerosis. If an individual is identified with several risk factors then his chances of developing atherosclerosis are more than proportionally increased. Other conditions predisposing to the disease that have not been environmentally implicated include hemodynamics, blood coagulability, immunological make-up, genetic factors and obesity. Most of the risk factors which have been correlated with atherosclerosis and CHD do not correlate with stroke or cerebral vascular change.[5-7] Hypertension, however, is associated with stroke[5-7] and control of blood pressure in a long-living population is a major medical problem.

Although the effect of diet on atheromata formation is well documented and in fact diet is the primary therapeutic approach, many of the atherosclerosis investigators are debating the usefulness of dietary prevention on a national scale. Long-term studies such as those in Chicago, the New York Anti-Coronary Club, National Diet Heart Study and the Los Angeles Veterans Administration show that a dietary regimen low in fat drops serum lipid levels and somewhat lowers the incidence of fatalities. Whether similar diets are practical with large populations is the subject of the current controversy.[8,9] Noted researchers, including Stamler, Dayton and Malmros, have concluded that modification of the national diet is critical while others such as Kritchevsky are of the opinion that the stress resulting from adherence to an unfamiliar diet would override any benefit obtained from the more favorable food intake. Page[10] recommends a massive dietary study funded and conducted by the government to further clarify the situation.

Combination of foods, specific foods or lack of them and frequency of eating have all been discussed during the past year.[11-23] Sucrose has been cited as a contributing factor to atherosclerosis by a number of authors[11-16] but questioned by others.[17,18] Little and coworkers[11-13] showed conclusively that sucrose feeding elevated serum lipids of patients on a typical North American diet (high in animal or saturated fat)

but did not affect serum lipids of patients on a low cholesterol unsaturated fat intake. When starch was used as the carbohydrate source instead of sucrose, there were no effects with either diet.

Several investigators have suggested that a specific dietary deficiency influences atheromata formation. Substances cited for this effect include ascorbic acid,[19] linoleic acid[20] and chromium.[21] The linoleic acid evidence is based on the increase in atherosclerosis since the turn of the century and the concomitant decrease in linoleic intake due to hydrogenation and the use of prepared foods. Moreover, linoleic acid is the biosynthetic source of prostaglandins, important regulators of body reactions. Similarly, chromium, a constituent of raw sugar and necessary for glucose utilization, has been lessened in the diet by the refining of sugar.

Corroboration of previous findings relating to various risk factors (e.g. exercise, smoking, etc.) were obtained during the year.[6,7] In stress, Friedman et al.[23] found that tense, driving individuals had higher levels of serum lipids and a significantly greater insulin response to glucose than a more relaxed group while several authors observed that forced exercise in rats increased lipid metabolism.[24,25] Kannel[26] has postulated that exercise is important in preventing CHD because it promotes collateral circulation and lessens the effect of myocardial infarction. He suggests a national program to increase physical activity. Cigarette smoking and the resulting hypoxia were again noted as influencers of atheromata development.[27-30]

The current concept of atherosclerosis formation postulates that arterial injury occurs first followed by a sequence of events culminating in plaque deposits. The two prevalent theories of plaque pathogenesis are the infiltration concept and the thrombogenic theory. They involve different mechanisms and are not easily reconcilable. The filtration theory presumes that the plasma constituents which normally diffuse into the vessel wall from the luminal surface are localized at the injury site initiating plaque formation. In the other major theory, mural thrombi adhere to the injury surface and become incorporated into the wall by an overgrowth of endothelium. The type of plaque formed is dependent upon the ratio of the adhering materials, platelets and fibrin.

Murphy and coworkers[31] presented an interesting paper in which they describe an experiment with rabbits that suggests the initial injury to the vessel can be immunologic in nature. In their study, rabbits subjected to allergic injury (horse serum) developed coronary atherosclerosis similar to man while control rabbits, even on a high lipid diet, did not develop

plaques. The extent of the fatty deposits in the allergen-treated animals increased with increasing degree of dietary lipid. Immunological blood grouping[32] and complement[33] have also been mentioned as possible factors in the atherosclerotic process.

Additional factors discussed as possibly influencing this disease include air pollution,[34,35] serum iron level,[36] aortic proline hydroxylase[37] and heart rate.[38] One of the more original concepts of atheromata development in 1970 is the postulation by McCully and Ragsdale[39] that an aberration of homocysteine metabolism may play a role in plaque formation. Their theory is based on several points, namely, that (a) homocysteinuria is associated with accelerated atherosclerosis, (b) homocysteine added to normal cell cultures produces proteoglycans which have been implicated in atherosclerosis and arterial elastin damage, (c) methionine, the precursor of homocysteine, is found predominately in meat and dairy products, and (d) administration of homocysteine to rabbits for a five-week period produced vascular lesions similar to that found in early atherosclerosis.

Diagnosis - Several new procedures have been advocated for the diagnosis of CHD prior to the appearance of clinical symptoms. Doyle and Kinch[40] were able to identify ischemic heart disease in an ECG test following exercise. Over a five-year period, 85% of those showing abnormal ECG developed CHD (either angina or myocardial infarction). If verified, this procedure would not only be useful in identifying clinically unknown atherosclerosis but also might be useful in evaluating efficacy of CHD treatment. Page and coworkers[41] find a correlation, 0.90 probability, between CHD, age, total cholesterol and triglyceride while Lees[42] is developing immunoassay techniques for the rapid identification of blood lipoproteins. Ultrasonic measurements continue to be explored as potentially valuable in the estimation of arterial damage.[43]

Therapy - The prevailing therapeutic approach to atherosclerosis focuses on the treatment of hyperlipidemia, hypertension, obesity, diabetes and other associated pathologies. At present there is no extensive program for regression of plaques. This review will confine itself to the treatment of hyperlipidemia; the related diseases are discussed in other chapters.

There are two major indications for the lowering of serum lipids. One is the reduction of severe hyperlipidemia to (a) prevent lipid deposits (xanthomata) that can be disfiguring and occasionally painful and (b) elimination of abdominal pain and pancreatitis due to high lipid levels. The other rationale to reduce serum lipids is the strongly

suggested but as yet experimentally unproved hypothesis that lowering of these blood constituents will lessen the likelihood of CHD and atherosclerotic risk.

Clinical management of hyperlipidemia has been discussed in two well written manuscripts by authors with extensive experience.[44,45] Their concepts should be borne in mind by all researchers in the atherosclerotic drug field. Proper dietary management is the primary approach and is essential to successful therapy. In fact most therapeutic failures are due to the inability of the patient to follow the prescribed diet. The diets used are directed toward reduction of obesity and replacement of meat and other saturated fat products with foods containing unsaturated fats and nonmeat high protein substances.

If food management does not lower serum lipids sufficiently, the levels may be decreased further by administration of hypolipidemic drugs. Drug therapy at the NIH clinical center has been outlined by Levy and Fredrickson[45] and is shown in the following table:

Hyperlipoproteinemia	Drug of Choice
Type I	no effective drug at present
Type II	cholestyramine, D-thyroxine, nicotinic acid
Type III	clofibrate, D-thyroxine, nicotinic acid
Type IV	clofibrate, nicotinic acid
Type V	nicotinic acid, clofibrate

The regimens described by Lees[44] and Casdorph[46] are similar although the former also uses neomycin and β-sitosterol for patients with Type II hyperlipoproteinemia. Kuo[48] agrees with this therapeutic approach although he stresses alleviation of chronic overnutrition, particularly carbohydrates, which may lead to poor lipid clearance, hyperinsulinism and glucose intolerance.

As unsaturated fats have been known for some time to be helpful in reducing plasma cholesterol levels, most dietary regimens stress the intake of foods containing this class of compounds. Using the sterol balance study technique, Grundy and Ahrens[48] found that unsaturated fats do not affect sterol excretion, absorption or biosynthesis but act by causing redistribution of cholesterol into tissue pools. There is some as yet inconclusive evidence that the cholesterol redistributed into the tissues is excreted secondarily in the form of

either bile acids or neutral steroids. In view of the importance now given to unsaturated fats, it is imperative that further data be gathered to fully evaluate the significance of these findings.

Atherosclerosis chemotherapy has centered on compounds or substances that will lower specific serum lipids, notably cholesterol and/or triglyceride. In addition to the drugs already commercially available for a number of years (clofibrate, nicotinic acid, cholestyramine, β-sitosterol, D-thyroxine, neomycin and various estrogens), a number of new and old compounds were cited during the year for their lipid lowering and antiatherogenic properties.

An extensive study of clofibrate (2-[p-chlorophenoxy]-2-methylpropionic acid ethyl ester) manifested itself in over 100 publications in 1970 which mentioned this drug. Most of the work, however, confirms previous findings and is of little interest except for those compiling compendia. Vester and coworkers[49] discuss their experience with clofibrate in the treatment of diabetic patients over a period of five years. The drug lowered serum cholesterol and triglyceride below starting levels and maintained the lower concentrations throughout the study. Over thirty other laboratory measurements were monitored during the treatment period, and none showed more than a transitory change. The triglyceride-lowering action of clofibrate was variously attributed to decreasing production and accelerating clearance,[50] stimulation of adipose lipoprotein lipase,[51] reduction of tissue adenyl cyclase[52] and to inhibition of acetyl coenzyme A carboxylase.[53] Clofibrate was found to inhibit intestinal as well as hepatic cholesterogenesis in the hamster.[54] Care in the indiscriminate use of clofibrate, or any drug, without accompanying laboratory measurements was underscored by Wilson and Lees[55] who found that in several patients clofibrate reduced very low density lipoprotein cholesterol but raised the cholesterol content of the low density lipoprotein fraction.

Of the many clofibrate analogues that were described for use in atherosclerosis during 1970, nafenopin (Su-13437, 2-methyl-2-[p-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxy]-propionic acid) was studied the most extensively.[56-62] This drug was found to be effective in reducing both serum cholesterol and triglycerides. The latter activity was illustrated by Weiss *et al.*[56] who reported a 68% decrease in triglycerides of hypertriglyceredemic patients and by Duncan and Best[57] who reported a 51% decrease of this blood lipid in similar patients. Comparative studies of clofibrate with nafenopin[58,59] suggest that the newer compound is the more potent and in addition showed considerable activity in Type II

hyperlipoproteinemia.[59] CLY-503 (1,3-propanediol bis[α-p-chlorophenoxyisobutyrate]), another clofibrate analogue, was reported to be less toxic and more active in the reduction of serum cholesterol than the parent compound.[63,64]

Agents affecting cholesterol absorption by reaction with bile acids continue to be investigated. Neomycin whose cholesterol lowering activity was previously attributed to its antimicrobial potency was the subject of several manuscripts.[65,66] The authors of these papers concluded that neomycin acted not as an antibiotic but rather by selectively precipitating bile acids. Cholestyramine, a resin which lowers intestinal bile acid by ionic binding, has stimulated research for a more active synthetic. One material currently undergoing clinical evaluation is colestipol (U-26, 597A), an insoluble copolymer of tetraethylenepentamine and epichlorohydrin. Hypolipidemic effects of this substance have been shown in man[67,68] but further study, particularly on a comparative basis with cholestyramine, is needed. The cholesterol-lowering action of N-(α-methylbenzyl)linoleamide was reported to be mediated through inhibition of sterol absorption.[69]

Combination of drugs with different lipid-lowering mechanisms may be an area of future activity although there are inherent problems in this approach. Of interest is the paper by Samuel and coworkers[70] who found that neomycin plus clofibrate was more effective in reducing cholesterol than administration of either drug singly.

Probucol (DH-581, 4,4'-[isopropylidenedithio]bis[2,6-di-t-butylphenol]) lowers serum cholesterol in mice, rats and monkeys without affecting serum triglycerides.[71] Since this substance represents a new class of hypocholesteremic compounds,[72] it would be of interest to determine its clinical usefulness in extended studies.

N-γ-phenylpropyl-N-benzyloxy acetamide (W-1372), a hypocholesteremic which also moderates aortic lipid formation, was shown to reduce lipid deposits in the heart of rats maintained on a high lipid diet.[73] The drug also affects cholesterol oxidation in rat mitochondrial preparations.[74] Pyridinolcarbamate, an old compound reported to dissolve cholesterol deposits in arterial walls and promote regeneration of damaged arteries, was evaluated by Shimamoto et al.[75] in 43 patients suffering from atherosclerosis obliterans. These investigators found significant improvement in blood flow, mobility and related symptoms. The preliminary results with these compounds are particularly encouraging since few substances are known that will relieve atherosclerotic symptoms after development. It is possible that

agents with this type of activity combined with hypolipidemic drugs will be the therapy of the future.

Additional substances of interest which were cited in the literature for their effect in atherosclerosis or related conditions include N,N-dimethyl-N'-(4-phenoxyphenyl)sulfamide (U-25,030),[76] 2,2'''-/(1-methyl-4,4-diphenylbutylidene)bis(p-phenyleneoxy)/bistriethylamine (SQ-18,576) and its salts,[77] 4-(3,4-dimethoxybenzyl)-2-imidazolidinone (RO 7-2956),[78] the terpene, alisol A-24-monoacetate,[79] 2-methyl-2-/p-(p-chlorophenyl)phenoxy/propionate,[80] cyclandelate,[81] chlorcyclizine,[82,83] phenobarbital,[82,83] reserpine and analogues,[84] chondroitin,[85] heparin and dextran,[86] lecithin,[87] tomatine,[88] lignin[89] and various other natural products.[90,91] Compound series studied include cyclohexane and indan derivatives[92] and alkylidenedithio bisphenols.[72]

Other therapeutic approaches that are used include ileal bypass which was reviewed by Gomes et al.[93] They suggest that this treatment should only be considered if conventional procedures described above are unsuccessful. Aorto-coronary bypass as an emergency measure has also been suggested with reservations for acute cases of myocardial infarction.[94]

Miscellaneous - One of the major problems facing researchers in the atherosclerosis field is the lack of a good laboratory model that resembles the human disease state. Several leads in this area were described during the year.[31,39,95] Kramsch and coworkers[95] reported that they induced severe coronary atherosclerosis with 60% narrowing of the arterial lumen in all of 40 Macaca irus monkeys fed a lipid diet for 18 months. The lesions resembled human disease in distribution and microscopic appearance. Similar deposits were also induced in rabbits by homocysteine administration[39] and by allergic injury.[31]

The 95-page report from the American Heart Association,[3] which includes discussion of long-term studies of antilipidemic drugs, provides contemplative reading for chemotherapeutic investigators. While the study commission acknowledges the efficacy of the known hypolipidemic drugs, their comment on long-term therapy warrants quotation: "What is yet to be determined is whether biochemical action of these or similar drugs will exert any favorable effect on the cause of the atherosclerotic diseases and whether long-term continued use of these substances produces significant deleterious effects." It should be borne in mind that this avenue of thought is consistent with probable government action on long-term evaluation of oral antidiabetic compounds.[96] A key study on this subject, the effects of lipid-lowering drugs in

postcoronary patients, is being carried out by 53 universities. The results of this investigation could establish trends for future clinical evaluation of hypolipidemic agents.

The study commission's basic approach to primary prevention of atherosclerosis in this country is threefold. They suggest a reduction of hypolipidemia and associated disease states by dietary restrictions, pharmacologic control of elevated blood pressure and elimination of cigarette smoking.

Comment - The reduction of serum lipids has now advanced to a functional clinical entity. Appropriate treatment of hyperlipidemia should become available on a routine basis as capability to perform phenotyping of hyperlipoproteinemias expands beyond its present limited environment of laboratories primarily devoted to lipid research.

Fields of atherosclerotic research which remain only partially tilled are the understanding of atheromata pathogenesis and the chemotherapy of lesion regression.

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Chapter 17. Steroids and Biologically Related Compounds

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I. REPRODUCTION

A. Female Contraceptives - Oral contraceptives of the estrogen-progestagen type continued to be attacked on the question of safety. Most untoward effects were associated with the estrogen component. Analysis of reports of thromboembolism in the U.K., Sweden, and Denmark led to the conclusion that the dose of estrogen correlated with the incidence of pulmonary embolism, deep vein thrombosis, cerebral thrombosis, and coronary thrombosis.[1] This led the Committee on Safety of Drugs in the U.K. to issue a warning that oral contraceptives containing more than 75 µg of estrogen may lead to thromboembolic episodes.[2] These conclusions were criticized.[3,4,5] It was pointed out that Conovid-E which contains 100 µg mestranol and 2.5 mg norethynodrel had a low incidence of thromboembolism while Volidan which contains 50 µg ethynyl estradiol and 4 mg megestrol acetate (MA) had a high incidence of thromboembolism. The Committee suggested that the nature and the dose of progestagen may also play a part in thrombogenesis. Oral contraceptives appeared to increase the risk in women to ischaemic heart disease.[6] Many papers support the contention that estrogens increase the risk of thromboembolism. Women using ethynodiol diacetate + 100 µg mestranol had decreased antithrombin III activity to levels similar to those of women in the third trimester of pregnancy.[7] Platelets isolated from women on different combined estrogen-progestagen contraceptives aggregated more rapidly.[8,9] Other reports suggested that estrogen-progestagen contraceptives were not substantially involved in thrombogenesis. In a study comprising 5,952 women using oral contraceptives (57,492 cycles), thrombophlebitis was reported at an incidence rate of 1.6 per 1000 women per year which is within the "normal" range.[10]

Many papers were published concerning other changes seen in women using the combination contraceptives including abnormal glucose tolerance tests[11,12,13,14,15] hypertension,[16,17,18] changes in concentration of the serum protein components,[19,20,21] decreased ability to utilize folate polyglutamates,[22,23] changes in plasma lipids,[24,25] psychological changes[26,27] and cutaneous side-effects.[28] The long-term clinical significance of these observed changes is not known.

Most adverse effects have been attributed to the estrogen component, making research with low dose progestagens all the more important. The latter type of contraceptive may decrease the number and severity of the untoward reactions seen with combination type contraceptives.[29,30,31] Injection of a depot progestagen, depot-medroxyprogesterone acetate (Depo-MPA) prevented pregnancy in women injected every three months with 150 mg.[32] Abnormal bleeding patterns resulted. These could be overcome with daily, oral administration of stilbestrol,[33] but the addition of estrogen would seem to remove any alleged advantages of an estrogen-free contraceptive. Norethindrone enanthate used in a similar way (300 mg every three months) proved to be an effective contraceptive,[34] without producing thromophlebitis or abnormal glucose tolerance. Although they were not compared in the same series of women, breakthrough bleeding did not seem to be as much of a problem as with Depo-MPA. Daily, oral doses of a progestagen

is another method of decreasing the amount of drug required for contraception. Chlormadinone acetate (CAP) can prevent pregnancy in women taking 500 µg each day.[35] This drug was removed from the market because in chronic toxicity studies beagles receiving up to 25 times the human dose developed breast nodules.[36] The significance of this finding can be questioned, since the occurrence of nodules was not dose related, no nodules have been found in other species including man, and CAP in sequence *with* estrogen has not produced nodules in beagles. Pregnancy was also prevented when MA was given orally on a daily basis.[37] Formulation of MA, 500 µg in peanut oil solution, enhanced the contraceptive potency.

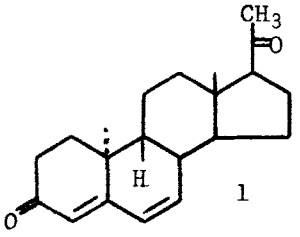
A major advance in drug delivery systems was attained through the use of silastic rubber implants which permit some drugs to be maintained at relatively constant blood levels over long periods of time. The silastic implant can be removed at any time to stop drug action. The initial rate of release may be significantly greater than the constant rate eventually achieved.[38] Progesterone diffuses too rapidly from silastic for a duration effect to be achieved. Norgestrel, MPA and CAP are all released slowly enough to bring about the desired effects for well over one year when administered in a 30 mm silastic tube. MA, 25 mg, in a silastic capsule was found to have a release rate of approximately 24 micrograms per 24 hours.[39] The vagina was explored as a possible site from which contraceptive drugs in silastic could be absorbed.[40] Silastic was molded into cylindrical rings ranging in diameter from 72 to 80 mm and containing 2 grams of MPA. The results show the drug is sufficiently absorbed from these rings to cause a rise in basal body temperature and to inhibit LH release. Removal of the silastic ring leads to rapid cessation of drug activity. Subsequent studies demonstrated that a constant release rate of 550 µg MA from a vaginal ring is sufficient to inhibit ovulation.[41] Lower doses of MA may also prove to be effective.

Attempts have been made to determine the mode of contraceptive action of progestagens. Depo-MPA inhibited ovulation presumably by inhibiting the preovulatory LH surge.[42] Laparotomies performed on women 2-6 months after progestagen injection showed that follicular growth and development, from primordial to Graafian stage, was not impaired. Oral microdoses of CAP did not inhibit ovulation as shown by culdoscopic visualization of the ovary demonstrating fresh corpora lutea.[43] In this study viscous cervical mucus correlated well with the antifertility effect of CAP. The drug at 100 µg/day caused similar cervical mucus changes with no contraceptive effect. Other investigations confirmed ovulation in women on microdoses of CAP but found lower than normal levels of progesterone possibly due to defective function of the corpus luteum.[44] Daily administration of norgestrel (50 µg) did not inhibit ovulation but did decrease the output of progesterone from the corpus luteum.[45] The amount of MA released from silastic capsules did not inhibit ovulation or prevent the normal development of the endometrium. Intracervical silastic tubing releasing 200-250 µg/24 hr of progesterone inhibited secretion of cervical mucus.[46] However, spermatozoa, obtained in a post-coital test, exhibited a fair degree of longevity despite the reduction in quantity, increase in viscosity, and apparent hostility of the cervical mucus. Other studies indicate that CAP and MA cause cervical mucus changes preventing penetration of the spermatozoa.[47] A study of infertile women pointed up the difficulty of correlating the physical

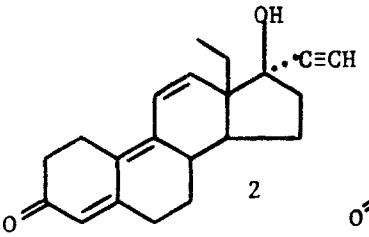
properties of the cervical mucus and the infertile state.[48] The mode of action of low dose progestagens in women is unclear and the most that can be said is that the drug usually acts after ovulation. CAP in rabbits[49,50] can interfere with fertilization, accelerate tubal transport of ova and produce ovicidal effects. Rats, receiving daily 0.3 µg of norgestrel or 1.5 µg of MA, orally for 12 months failed to come to term after a successful mating.[51,52] The only significant change was a depression of the metabolic status of the uterus which could have led to fetal resorption. It would be interesting to know how soon after the start of norgestrel or MA therapy the rats became infertile. In rats implanted with silastic tubing containing either progesterone or CAP, with release rates of 84.32 or 10.76 µg/day, respectively, fertility was not effected during a 45 day period.[53] Rhesus monkeys, implanted with silastic capsules releasing approximately 4-8 µg/kg of MA per day, showed irregular endometrial patterns.[54] The degree of lymphocytic and polymorphonuclear infiltrations in the endometrium was the most striking observation and this may be the mechanism of contraceptive action, i.e. release of leucocytic lysozomal enzymes.

The clinical use of low-dose progestagens produced few reported severe side-effects. Women with subcutaneously implanted silastic capsules containing MA have had few side-effects and no gross evidence of total tissue reaction from the silastic.[55] Norgestrel, at a daily dose of 50 µg, did not decrease glucose tolerance or raise serum transaminase levels.[56]

New contraceptives have been submitted for clinical trial. Dydrogesterone (1) combined with quinestrol was found to prevent pregnancy when given as a single oral dose on day 22 of the cycle.[57] The main drawback to this combination is that it causes an irregular menstrual pattern. This approach is not new, and is dependent upon the duration of activity of quinestrol which is stored in the body fat. This method of delivering drug



1



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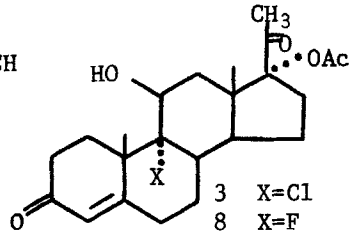
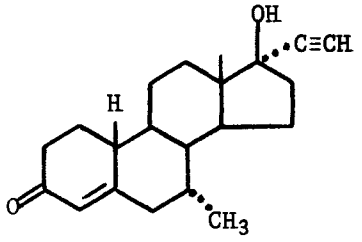


3 X=Cl
8 X=F

over long periods of time may be more desirable than depot injection but it does not offer the same advantage as the silastic vaginal ring where drug action can be terminated when desired. R-2323 (2) appears to have a biological profile similar to that of norethynodrel.[58] Compound 2 exhibits low estrogenic activity and progestational activity in the rabbit but does not maintain pregnancy in the castrated rat. It prevents pregnancy in women taking the drug orally, once-a-week, in contrast to norethynodrel which is given daily for 20 days in combination with estrogen. We hope clinical information will be forthcoming to show whether 2 is qualitatively or quantitatively different from norethynodrel. A new progestational agent, SQ 18,510 (3) has been reported to be a more potent anti-estrogen than CAP and may be useful as a low dose contraceptive.[59] U-13,851 (4) was described

as a compound with inherent estrogenic and progestational activity.[60] This compound may have potential as a once-a-week contraceptive if it does not induce withdrawal bleeding. It is well known that ethynyl estradiol (EE) in rats can cause fetal resorption if given after implantation. In women, EE at a daily dose of 1.0 and 5.0 mg for 7 consecutive days did not cause abortion[61] and it was concluded that EE may be effective as a post-coital contraceptive in women during a relatively short period following ovulation and just prior to implantation. Since estrogen treatment began 32 to 48 days following the first day of the last menstrual cycle, we cannot dismiss the possibility that EE could terminate a pregnancy immediately after implantation.

A number of non-steroidal agents have been reported to have post-coital antifertility action. A single 10 mg/kg oral dose of 2-phenyl-3-p-(β-pyrrolidinoethoxy)phenyl(2,1-b)naphthofuran administered immediately post-coitum prevented conception in rats, mice and rhesus monkeys.[62] The activity in monkeys can be questioned because the number of non-treated primates becoming pregnant was measured over a three-year period while the monkeys were kept on drug for only 6 months. A series of 2,3-diphenylbenzofurans, 5,6-polymethylene benzofurans and 1,2-diphenylnaphthofurans were found to have marked anti-nidation activity in rats.[63] Several basic ethers of 3-alkyl-2,3-diphenylpropiophenones (5a,b,c) prevented implantation in rats at a dose of 0.5 mg/kg administered for the first five days



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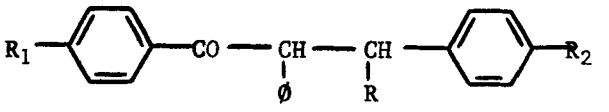
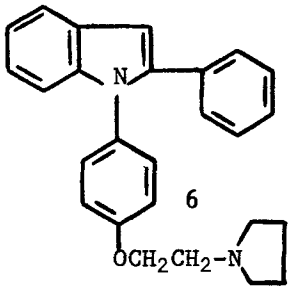


	R	R_1	R_2
5a	Et	$Et_2NCH_2CH_2O$	H
b	Et	$Et_2NCH_2CH_2O$	4-Cl
c	Me	$Me_2NCH_2CH_2O$	4-Cl

post-coitum.[64] Basic ethers of 1-(p-hydroxyphenyl)-2-phenyl-1,2,3,4-tetrahydroquinoline and 1-(p-hydroxyphenyl)-2-phenylindole were also shown to prevent implantation in rats.[65] Compound 6 was the most active in this series, preventing pregnancy at a dose of 12.5 mg/kg administered on the first 3 days post-coitum. Oral administration 2',3',5'-tri-O-acetyl-6-



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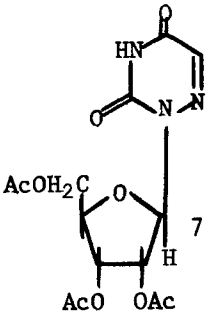

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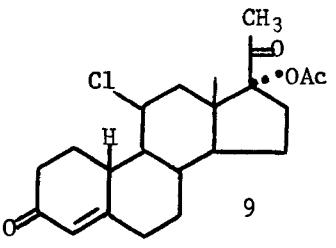


9

azauridine (7) at the very large dose of 300 mg/kg terminated the pregnancy in rabbits and rats.[66] Ovulation was not inhibited in either rat or rabbit. Dimenhydrinate had no anti-fertility activity but enhanced the contraceptive potency of estrogens in rats.[67] A constituent of the seeds Ensete superbum Cheesm, (VIDR-26D) prevented implantation in mice, rats, guinea pigs and hamsters.[68] This agent had no effect on the pregnancy in rabbits. The seeds of Abrus precatorius Linn. can prevent pregnancy in rats when given before and after mating.[69]

B. Ovulation Induction - The effect of clomiphene citrate (CC) on the ovulatory mechanisms received much attention this year. In rats, low doses of CC increased pituitary FSH concentration and the release of LH while high doses had the opposite effect.[70,71] Low dose stimulation of gonadotropin release is thought to be the effect seen in anovulatory women. CC (100 mg/day) was shown to cause a rise in LH levels in 3 out of 4 patients with normal pre-treatment gonadotropic levels.[72] The release of LH is thought to be sufficient to cause rupture of pre-existing ovarian follicles because there are many indirect signs of ovulation, e.g. increased basal body temperature, increased urinary pregnanediol levels and progestational changes of the endometrium.[73] There is, however, a discrepancy between the number of patients thought to have ovulated and the number known to have conceived. If one accepts as fact that ovulation has occurred, then inability to conceive may be due to at least four possible reasons: (1) unrecognized abortion, (2) inadequate luteal function, (3) accelerated tubal transport, (4) hostile cervical mucus.[73,74] It is also possible that these indirect determinants of ovulation are misleading and ovulation may not have occurred in all cases. Some postulate that production of progesterone in the unruptured follicle may be fairly common in patients taking CC.[75] This hypothesis is supported by the reports of follicular luteinization.[73,74,75] An additional possibility is that clomiphene, which has anti-implantation activity in rats, is preventing pregnancy after inducing ovulation.

The two isomeric components of CC were isolated and tested clinically.[76] Although both isomers are active, the cis form was more potent than the trans form.

C. Male Contraceptives - The developments in this area appear to have limited value for clinical usage. Methylene, ethylene, and propylene dimethanesulphonates were reported to effect different phases of spermatogenesis.[77] The qualitative differences between these drugs are interesting but their action is related to that of busulphan, an immunosuppressant. No further insights were developed as to the mode of action of 3-chloro-1,2-propanediol (U-5897).[78,79,80,81] U-5897 has been considered as a possible drug to limit the wild rat population by sterilizing the male.[78] Although the dose requirement may be high, the suggested approach appears to be novel. A metabolite of U-5897, 2,3-epoxypropane-1-ol (glycidol) was also antispermatogenic.[82]

D. Estrus Synchronization - Progestagens are still being tested as a method of synchronizing estrus in cattle. Long term injections of progesterone had different effects on follicular development, depending on the stage of the cycle when therapy was started.[83] The estrus cycle was shortened if drug was given for a few days early in the cycle but the cycle length was in-

creased if treatment was continued. The effect of melengestrol acetate (MGA) was studied in dairy cows and pregnant heifers.[84,85,86] MGA had no significant influence on the quality or quantity of milk. The cycles of the cows were synchronized although the fertility in the first controlled cycle was reduced. In rabbits, MGA inhibited sperm transport through the uterus, or decreased sperm fertility during uterine transit.[87]

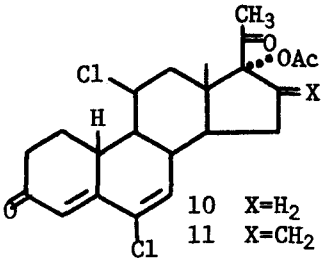
Progestagens are also being used to synchronize estrus in sheep. MPA was as effective in synchronizing the cycle in ewes when used in vaginal sponges or when placed in feed.[88] Decreased fertility in first controlled estrus has also been observed in ewes. Cronolone (8) caused post-treatment infertility either by impairing sperm transport and survival[89] or by inhibiting ovulation at the first estrus.[90] It would not be surprising if 8 also exerted corticoid-like activity. CC was also used as a possible ovulation-inducer after synchronization with cronolone pessaries.[91] CC in ewes acts as a weak estrogen, inducing estrus, and inhibiting ovulation. Estradiol was shown to be luteolytic in ewes, when used 9 days after ovulation.[92]

ICI 33828 (1-α-methylallylthiocarbamoyl-2-methylthiocarbamoyl-hydrazine) inhibits the release of gonadotropin and has been used to synchronize estrus in gilts. Those gilts, started on ICI 33828 during the follicular phase, came into heat but did not ovulate.[93] The drug may not synchronize ovulation well since more precise control was attempted with the use of gonadotropin.[94] Excretion and tissue distribution of ICI 33828 was studied in swine.[95]

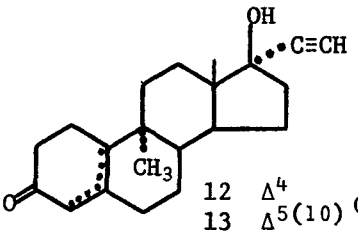
MPA was used to suppress estrus in bitches.[96] In addition to possible utility of oral progestagens in the "pet population," injected-MPA was used to limit the canine reservoir and vector for rabies. MA, which is being used orally to prevent estrus in bitches, is excreted rapidly from the animals.[97] Rapid elimination of MA may decrease the incidence of pyometria, a side-effect which caused the removal of MPA from the market.

II. PROGESTATIONAL AGENTS

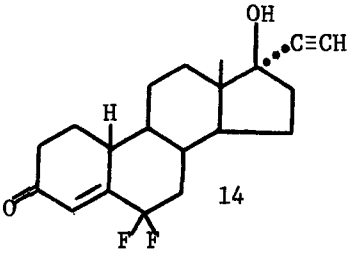
During the year several novel chemical structures with progestational activity were reported, some of them with potential use as contraceptives. The 11β-chloro-19-norsteroids 9 and 10 are among the most active progestational compounds so far reported.[98] The related 16-methylene compound 11



10 X=H_2
11 X=CH_2



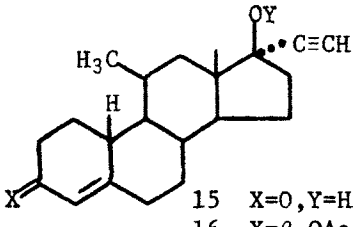
12 Δ^4
13 $\Delta^{5(10)}$



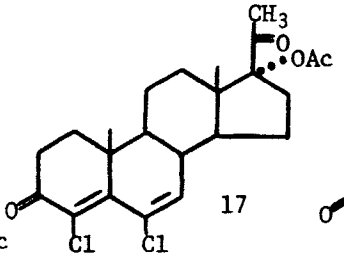
14

is 495 times as potent as progesterone (McPhail test, p.o.).[99] The Ψ-retrosteroids 12 and 13 were similar in activity to the 9-unsubstituted analogs, while 9α-methyl-19-norprogesterone had higher subcutaneous activity than progesterone.[100] The 6,6-difluorosteroid 14 was twice as active as the parent norethisterone in the McPhail assay and its oral anti-uterotrophic activity in mice was 3.9 times that of norethisterone.[101] The 11β-methyl-

steroids 15 and 16 exhibited potent progestational and anti-estrogenic activities, as well as estrogenic effects in the estrogen deficient state.[102]



15 X=O,Y=H
16 X=β-OAc,Y=Ac



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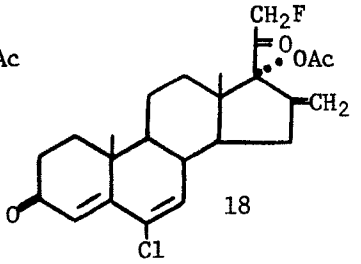
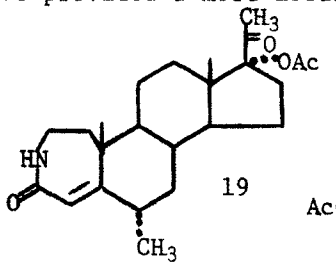


18

The 4,6-dichlorosteroid, Ro 7-2133 (17), was at least 50 times as potent as progesterone and showed weak anti-androgenic activity in castrated rats.[103] In vitro this compound is reported to lose the 4-chloro substituent and may be eventually converted to CAP.[104] The 21-fluorosteroid 18 was reported to be highly active in the Clauberg assay (105.4 x progesterone, i.m., 32.5 x p.o.).[105] The A-homosteroid 19[106] and 8α-methyl norethisterone[107] were among novel structures reported with interesting activities. In the case of compound 19 a detailed dose-response curve would have provided a more accurate comparison with the parent MPA. Clinically,



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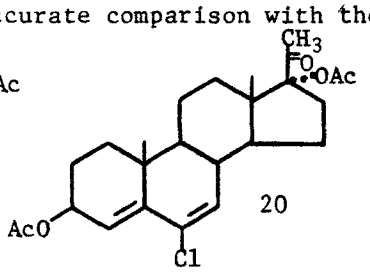

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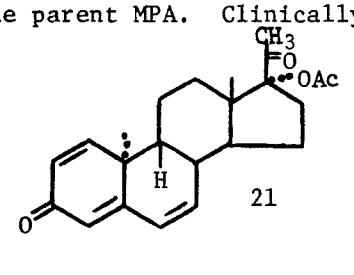
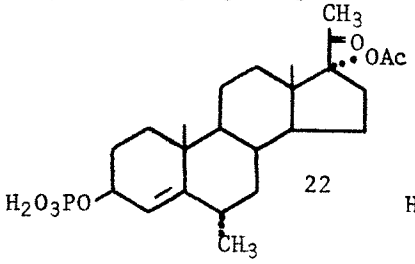


21

clogestone acetate (20) proved highly effective in achieving secretory endometrium and then inducing withdrawal bleeding on cessation of treatment.[108] Ro 4-8347 (21), a potent orally active progestagen, when given at the dose of 4 mg/day in the second half of the cycle, was found clinically useful in anovulatory women with decreased ovarian function.[109] The water soluble progestin 22, related to MPA, showed 10-100% of the progestational activity of the parent steroid in vivo and may be useful for intravenuous administration to prevent abortion.[110] It will be interesting to see if this compound is more effective in cases of threatened abortion than the orally active progestagens. Both isomers of 4-phenyl-1-acetylcyclohexanol



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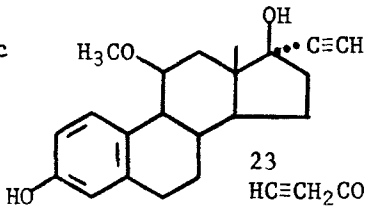

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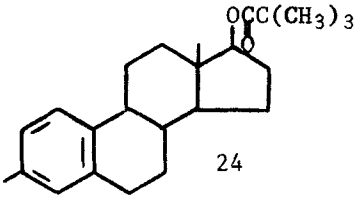

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exhibited significant progestational activities as measured by the carbonic anhydrase assay.[111]

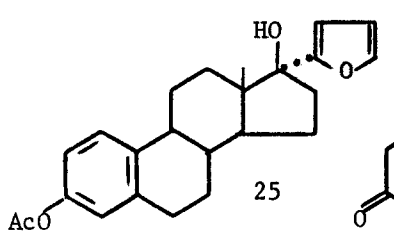
In three of eleven patients with endometrial carcinoma, 19-nor-17α-

hydroxyprogesterone caproate in oil, instilled directly into the uterine lumen, caused complete disappearance of the tumor.[112] The successful use of progesterone as an immunosuppressive agent in homologous skin graft in intact and ovariectomized female rhesus monkeys has been demonstrated.[113]

Progesterone was reported to induce drug metabolizing enzymes in the livers of mature female rats, but not in male rats.[114] This sex difference may be due to difference in endogenous androgen levels which stimulate hepatic enzymes in males. The urinary metabolites of the orally active progesterone-3-enol cyclopentyl ether in healthy subjects were the same as those of progesterone. The ratio of 5α- to 5β-metabolites increased seven fold, suggesting that the increased and prolonged activity of enol-cyclopentyl ethers of progestins may be due to metabolic differences.[115]

III. ESTROGENS

R-2858 (23), an orally active estrogen, has been found 5 times as potent as EE.[116] AY-20,121 (24), an estrogen with long duration of action, prevented



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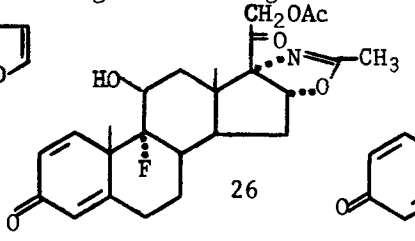

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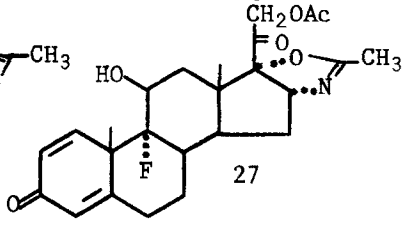

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conception in rats for a longer period than EE or quinestrol.[117] AY-11,483 (25) showed significant activity in the vaginal cornification test in rats and mice, but relatively weak uterotrophic effect in rats. This compound, like estriol, exerts a weak effect on the endometrium in rabbits.[118] The estrogenicity of estriol, estrone, and estradiol (between 0.001 μg to 0.1 μg) in lipoid solvent were approximately the same in mice. In doses of about 0.1 μg, aqueous estriol was significantly less effective than estriol in peanut oil.[119]

The use of quinestrol in postmenopausal women was reported.[120,121,122] The drug was well tolerated and caused minimal changes in the endometrium when given daily.[122]

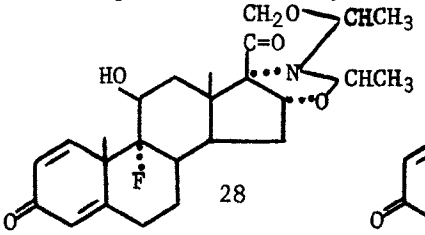
The biological potency of steroid hormones in rats was greater with drug placed in silastic implants than when injected s.c. in an oil vehicle. There was a more pronounced increase in potency with androgens and progestagens than with estrogens.[123]

Carneau pigeons spontaneously develop aortic atherosclerosis, similar to that seen in man. Daily administration of estrogens up to 26 months did not enhance the development of coronary atherosclerosis in any of these animals.[124]

IV. CORTICOIDS

L-6400 (26), in rats is a potent anti-inflammatory agent and exerts an effect comparable to that of dexamethasone not only on inflammation, but on some of the biochemical parameters (weight gain, food intake, nitrogen and electrolyte balance). Its effect on the pituitary-adrenal axis and on blood sugar in response to a glucose load is less intense and of shorter duration than that of dexamethasone.[125] In man, 26, used as 0.1% cream,

showed comparable activity to that of triamcinolone acetonide (0.1%) or



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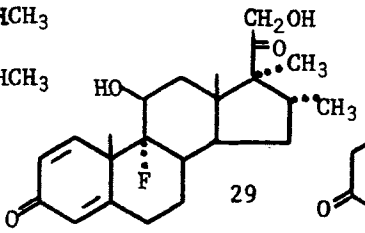

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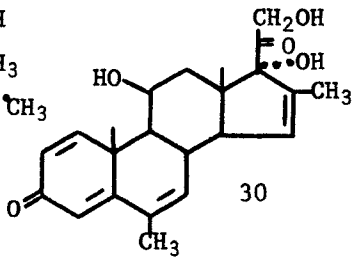

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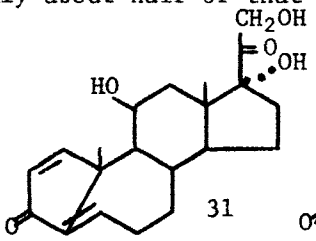
flucinolone acetonide (0.025%).[126] The isomeric [16α,17α-d]-oxazolino steroid 27 was also prepared but it was less active than 26.[127] The related hexacyclic corticoid 28 had high topical, as well as high systemic, activity in the rat.[128] Other novel corticoids with high activity were dimesone (29)[129] and the fluorine-free 30.[130] The latter compound had anti-exudative and anti-proliferative potencies equal to dexamethasone, while the gluconeogenetic and adrenal weight suppressive activity of 30 was only about half of that of dexamethasone. 10 (5→4) Abeoprednisolone (31)



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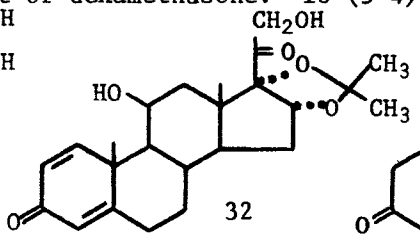

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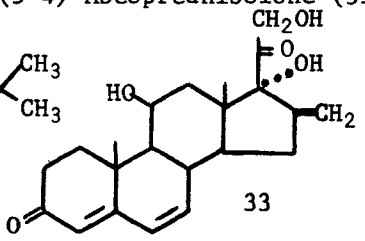
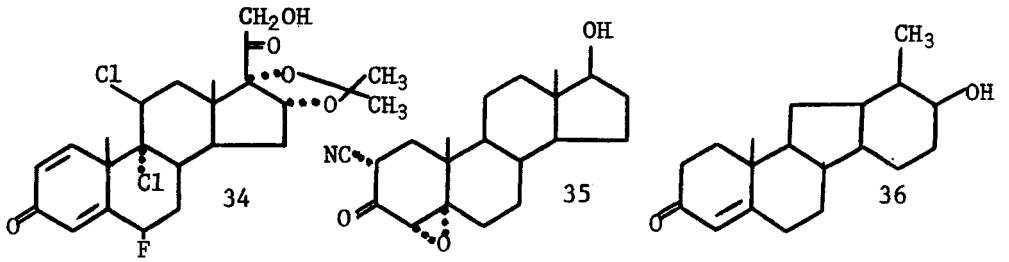


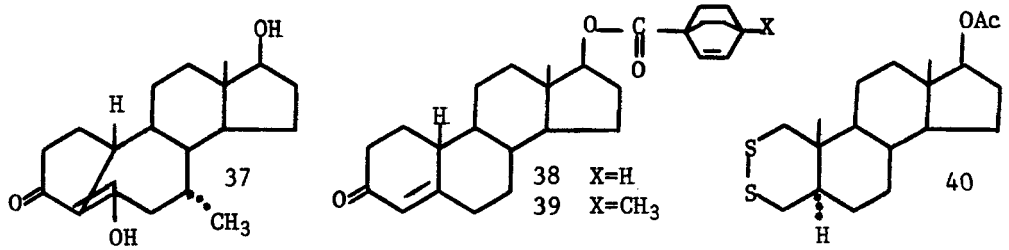
33

was reported to possess anti-inflammatory activity, but lower than that of the parent prednisolone.[131] Prednacinolone (32) is reported to show significant thymolytic, gluconeogenetic and topical activities in the potency range of triamcinolone acetonide,[132] and was clinically efficacious as a 0.1% ointment.[133] In a double blind trial in psoriasis, hydrocortisone 17-butyrate (0.1%, o/w cream) under plastic occlusion was as effective as triamcinolone acetonide (0.1% same vehicle). The authors suggest that under the conditions employed the systemic effects of 0.1% hydrocortisone 17-butyrate cream may be less than those of 0.1% triamcinolone acetonide cream.[134] This observation indicates again that esterification at C_{17} enhances the topical activity of most corticoids, even those with relatively low potency. The 17-phosphates of some potent corticoids (prednisolone, betamethasone) were prepared for the first time, but were almost entirely devoid of hormonal activity.[135] It appears that the 17-phosphate group renders the molecule inactive and there are no 17-phosphatases to effect hydrolysis. 7-Dehydroprednisolone has anti-inflammatory activity but no firm data were reported.[136] Papavallarinol (3β-methylamino-5-pregnene-18,20-diol) exhibited anti-inflammatory activity in intact rats but not in adrenalectomized animals. This compound inhibited histamine-induced ulcers in Shay rats.[137] The triterpene derivatives, sodium nimbinate and hederagenin, exhibited significant anti-inflammatory activities (carrageenan paw).[138] Isoprednidene (33) was studied in hirsute women with oligomenorrhea or secondary amenorrhea and elevated excretion of 17-ketosteroids. In 30 of 39 patients at dosages of 5-10 mg/day normal excretion of 17-ketosteroids



was observed. During therapy hirsutism decreased in only 2 women, but menstrual cycles became normal in 21 women and 4 became pregnant.[139] Although no progestational activity was reported, such activity could account for regularization of cycle in half of the patients. Fluclorolone acetonide (34), a potent topical anti-inflammatory steroid, had negligible effect on bone. In contrast to these findings flumethasone (a 6α,9α-difluorosteroid) had low topical activity but suppressed bone growth and caused osteoporosis in rabbits.[140]

The antiasthmatic action of corticoids and their mechanism of action was reviewed.[141] The use of dexamethasone acetate (i.m. suspension) in the treatment of perennial allergic rhinitis resulted in good to excellent relief of symptoms in 75% of the patients.[142] A series of cyanoketones related to 35 were shown to block catabolic and thymolytic responses to exogenous ACTH in castrated male rats.[143] Such compounds may inhibit the synthesis or release of adrenal corticoids. Several C-nor-D-homosteroids related to 36 exhibited marked anti-aldosterone activities.[144]

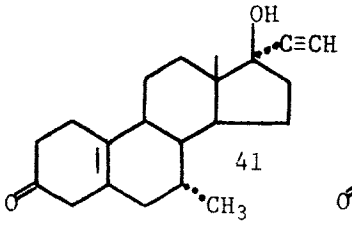


Several catatoxic steroids were studied and were found to protect animals against the toxic actions of numerous drugs. The most active catatoxic steroids are spironolactone, ethylestrenol and norbolethone. Thus, spironolactone offers protection against cardiac necrosis, convulsions and mortality induced by digitalis.[145] Ethylestrenol, spironolactone, and prednisone (the last is not a catatoxic steroid) protected rats against heavy overdoses of meprobamate.[146] The increase in nonspecific resistance due to catatoxic steroids appears to be unrelated to anti-mineralocorticoid potency.[147] Probably many, if not all, of these protective effects are due to the induction of hepatic microsomal drug-metabolizing enzymes by the catatoxic steroids.[148] Most of the catatoxic steroids possess a 17β-oxygen function as well as a 17α-alkyl function. These moieties are known to increase the effect of steroids on liver function.

V. ANDROGENS

In man, 19-nortestosterone 17-dodecanoate (100 mg, once a month) was found to be a potent, long acting anabolic agent.[149] Ba-36,644 (37) had greater

anabolic activity than stanozolol in animals as measured by effects on muscle ribosomes and bone histology.[150] The androgenic, myotrophic, and antigonadotrophic properties of 13 clinically used anabolic steroids were



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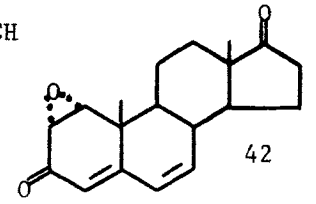

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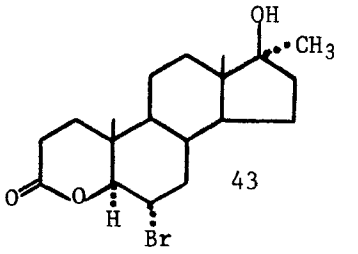

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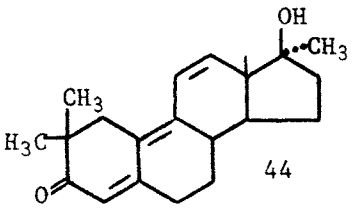
compared in rats. The data indicate that chemical alterations in androgenic steroids can result in separation of androgenic from anabolic activity, while a separation between anabolic and antigonadotrophic properties has not been achieved.[151] A series of esters of 19-nortestosterone with 4'-substituted or 4'-unsubstituted bicyclo[2,2,2]octane carboxylic acids were examined in the rat for their duration of anabolic activity. The esters 38 and 39 are exceptionally long-acting anabolic agents with low androgenic activity.[152] The dithiasteroid 40 had significant androgenic activity in the rat.[153] The 7α-methylsteroid 41 is both anabolic and estrogenic and was tested clinically against skeletal disorders.[154] In seven patients with senile osteoporosis, compound 41 caused a positive nitrogen balance in all patients, positive calcium balance in four patients (no



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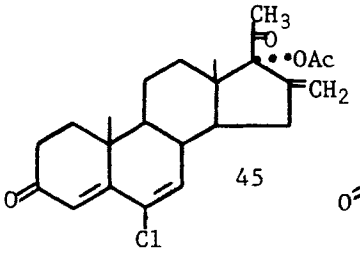

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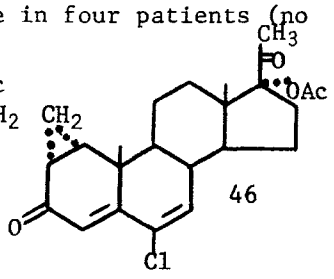

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change in three), and little change in phosphorus balance.[155] In man, 19-nortestosterone-3-(4-hexyloxyphenyl)-propionate is a potent anabolic agent. It showed no toxic side-effects on liver or kidneys and had no effect on blood electrolyte balance.[156] Norbolethone was found to be an efficacious and safe drug in stimulating linear growth in stunted children. There were no adverse effects on liver function and no evidence on accelerated epiphyseal fusion.[157] The use of testosterone in 5 male patients with Klinefelter's syndrome caused advance in bone age with all subjects, while decreasing gynecomastia; gynecomastia recurred after cessation of therapy. The investigators suggest testosterone therapy during adolescence and not in the pre- or post-adolescent period.[158]

The use of ethylestrenol combined with phenformin to reduce platelet stickiness in 9 arteriopathic patients was reported.[159] The epoxide 42, although devoid of anabolic-androgenic activity, possessed hypolipemic activity in the rat.[160]

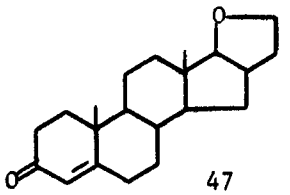
The erythropoietic effect of testosterone cyclopentylpropionate (TCP) was studied in the rat and found to be associated with the kidney.[161] In mice, several C_{21} steroid metabolites with the 5β-configuration stimulated

the incorporation of Fe^{59} into circulating erythrocytes, and those with 5α-configuration had no effect. The most active 5β-metabolite was 5β-dihydroprogesterone.[162]

VI. ANTI-ANDROGENS

The major thrust in this area was not on the synthesis of new compounds, but on the development of new assays and application of known androgen-antagonists to new uses.

The 4-oxasteroid 43 exhibited anti-androgenic activity (1 mg/day, s.c.) in rats.[163] MA, melengestrol acetate (MNA), and dimethisterone (DM),



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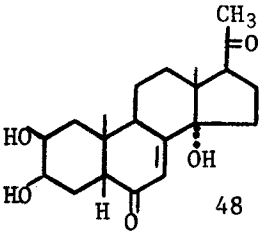

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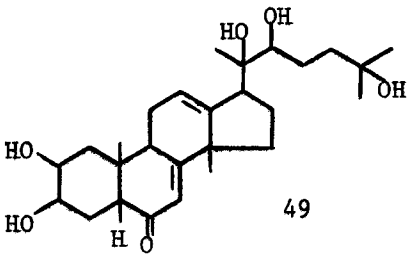
three progestational agents were compared for their anti-androgenic activities. While the progestational activities in the rabbit were MNA>MA>>DM, their anti-androgenic activities in castrated mice (s.c.) were DM>MNA=MA.[164] The potent progestin, Sch 12600 (45) and CAP were compared for their anti-androgenic activities in several species. Only 45 was effective in reducing the secondary sex organ weights in intact rats. It also produced a feminization of male fetuses, as measured by a reduction in the anogenital distances, when administered to pregnant rats. Both compounds were found to be effective in reducing the size of the prostate in aged dogs with benign prostatic hyperplasia (BPH) but 45 was more effective.[166] A comparison in the chick comb assay of six anti-androgens, including two dodecahydrophenanthrene derivatives, showed that cyproterone acetate (46) was the most potent.[167] The tetrahydrofuran 47[168] and 7-hydroxy-7,8,9,10-tetrahydrobenzo(c)-phenanthridine[169] demonstrated anti-androgenic activities in castrated rats.



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A novel in vitro method to measure anti-androgenic activity has been developed. This assay measures the inhibition of uptake of tritiated dihydrotestosterone (DHT) by various anti-androgens in rat prostatic tissue. Although the assay has the advantage of requiring small amounts of test compound, the structure-activity correlations are quite different from those obtained from intact animal assays.[170]

Methods for chemotherapeutic treatment of BPH were briefly reviewed.[171] It was demonstrated that although the concentrations of testosterone and androstenedione do not differ between the normal and hypertrophic glands,

there is a 5-fold increase in the concentration of DHT in hypertrophic as compared to normal glands. A tentative hypothesis was advanced that the accumulation of DHT in the human prostate may be causally related to the development of BPH.[172,173]

Cyproterone acetate (46), the most widely studied anti-androgen, failed to effect androgen-dependent aggressive behavior in mice. It was concluded that 46 probably does not block the androgen-receptor in the central nervous tissue in the same way as it does in non-neural tissue.[174] A 3-year study in 10 men receiving 46 (100-200 mg/day) indicated no impairment in adrenocortical function, although ejaculate volume and libido were severely depressed during the first year of treatment. The ejaculate volume began to increase after 17 months and 2 of 10 men were able to inseminate their spouses.[175] It was suggested, that anti-androgens like 46 may have some therapeutic value in the treatment of precocious puberty and adrenogenital syndrome.[176]

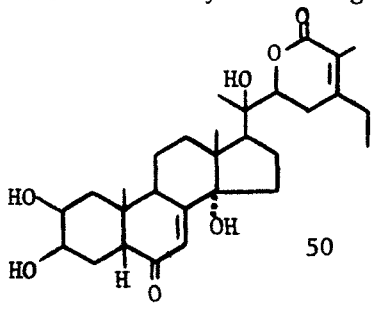
CAP in high doses (40-60 mg/day, p.o.) and 17α-methyl-B-nortestosterone applied topically significantly decreased sebum production.[177] The paired costovertebral organs of the hamster is used in an assay to distinguish direct topical activity from activity due to systemic effect. In this assay topically applied CAP, 19-nor-CAP, and Δ^1-chlormadinone-16,17α-acetonide were completely inactive. Further, Δ^1-CAP and 46 showed activity but it is questionable whether it was a direct "end" organ effect.[178] It appears that topical treatment of acne with steroidal anti-androgens is still far from realization.

VII. CARDENOLIDES

3-*Deoxy*digitoxigenin, prepared for the first time, compared to digitoxigenin had similar carditionic activity on the isolated frog's heart. This indicates that the 3β-hydroxy group is not an indispensable requirement for the activity of a cardenolide.[179,180] Strophantidin-β-l-arabinoside, a new semi-synthetic cardiac glycoside, can be used as a substitute for ouabain for rapid digitalization by the intravenous route.[181] The chemistry and pharmacology of cardiac glycosides and aglycones was reviewed.[182,183]

VIII. INSECT HORMONES

Poststerone (48), a C_{21} steroid, was isolated for the first time from natural sources. This compound is one of the missing links between a C_{27}-insect-*metamorphosing* steroid (cf. ecdysterone) and the C_{19}-compound, rubrosterone. Poststerone is inactive in the Calliphora test, but it induces adult development of the brainless pupae of the silk moth (Samia cynthia).[184] Among other novel phytoecdysones reported during the year are stachysterone-A (49), the first naturally occurring C_{27}-steroid with a rearranged methyl group, and



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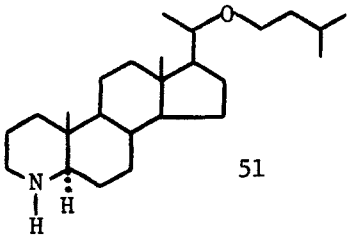

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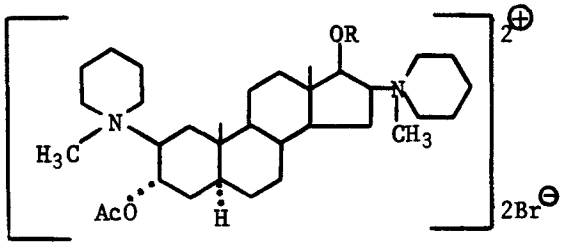
stachysterone-B, a 14-dehydroecdysone derivative.[185] An antiecdysone, ajugalactone (50) was isolated from plant sources, and its structure elucidated. It inhibits moulting of Chilo suppressalis (rice-stem borer) according to the dipping method.[186] The defensive substances of land and water beetles, many of them known to be steroids, were reviewed.[187]

IX. MISCELLANEOUS

The 4-aza-22-oxasteroid 51 in vitro showed good anti-microbial activity against C. albicans and S. aureus.[188] Paecilomycerol, a steroid with unpublished structure, showed strong antiviral activity in vitro with relatively low cytotoxicity.[189]

Pancuronium bromide (52), (erroneously reported last year as an i.v.



52 R=Ac
53 R=H

anaesthetic) and the related dacuronium bromide (53) were reported to have potent neuromuscular blocking action.[190,191] CT 1341, a new steroidal anaesthetic (a 3:1 mixture of 3α-hydroxy-5α-pregnane-3,20-dione and 3α,21-dihydroxy-5α-pregnane-3,20-dione 21-acetate) produced immediate induction of anaesthesia of short duration when injected into experimental animals. The recovery was rapid and uncomplicated.[192a] The animal data were also verified in man.[192b]

25-Hydroxyergocalciferol, a metabolite of vitamin D_2 was 1.5 times as effective as either vitamin D_2 or D_3 in curing rickets in rats.[193] 25-Hydroxydihydrostachysterol$_3$ was synthetized and isolated in pure form. This compound has weak antirachitic activity, but is a potent calcium mobilizing agent. Its biological activity suggest that it may be the drug of choice in the treatment of hypoparathyroidism and other similar bone diseases.[194]

X. REVIEWS

During this year some important reviews related to regulation of fertility were published. These dealt with the following subjects: physiology of early pregnancy,[195] aspects of fertility control,[196] antifertility agents,[197,198] antifertility agents in the future,[199] treatment of infertility,[200] induction of ovulation.[201] A review of estrogen metabolism in the diseased liver[202] and a historical review of glucocorticoids[203] were also published. The psychoendocrine aspects of breast cancer were reviewed.[204]

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Chapter 18. Non-steroidal Antiinflammatory Agents

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Introduction - This review will attempt to cover the highlights of research in the area of non-steroidal antiinflammatory agents (NAA), with an emphasis on work relating to the chronic arthritic diseases. Although the goal of this research is the discovery of drugs which will arrest, or even reverse, the inflammatory processes, the search also continues for safer, better tolerated drugs which will at least slow the processes and alleviate the disease symptoms. Several broad reviews of the area have appeared in 1970.[1-5]

Etiology and Pathogenesis - In spite of new arguments[6,7] for mycoplasma as the cause of rheumatoid arthritis (RA), the etiology of the disease remains uncertain.[8-10] A brief review article on the pathogenesis of joint inflammation in RA has appeared.[11]

Pharmacological and Biochemical Aspects - There have been few notable developments in the past year in *in vivo* methods used to screen for and evaluate NAA. Increasing reliance is being placed upon the adjuvant-induced arthritis assay in the rat since it is felt that the histochemistry[12] and pathology of this autoimmune disease most closely resemble the histochemistry and pathology of some of the human arthritic diseases. An improved quantitative method that employs the dog has been reported[13] in which the pressure exerted by the dog paw is measured after injection of an inflammatory agent into the corresponding knee joint. An insight has been provided into the 6-sulfanilamidoindazole-induced arthritis model in the adult rat through a study of the histological, chemical, and hematological events.[14]

In recent years there has been a growing awareness of an interrelationship between inflammation and the blood clotting processes. NAA have been shown to be effective as inhibitors of platelet and erythrocyte aggregation, as fibrinolytic agents, and as inhibitors of the release of damaging lysosomal enzymes from leucocytes. A central theme of membrane stabilization underlies many proposed explanations as to how the compounds work in these different areas.

Intrazole (BL-R743), aspirin, and phenylbutazone effectively inhibited rabbit platelet aggregation in both *in vitro* and *in vivo* screens.[15] Human blood platelet aggregation was inhibited by aspirin[16-18] and other NAA.[17-18] In all these cases the compounds are considered to work by inhibiting the release mechanism.

NAA inhibited macromolecule-induced aggregation of rat erythrocytes *in vitro*,[19] and at therapeutically active concentrations stabilized human erythrocyte membranes.[20] The fact that NAA inhibited denaturation of serum albumin by heat[21,22] focuses attention on the effects drugs may have on changes in protein conformation, and suggests that at least acidic NAA

may inhibit dog erythrocyte hemolysis by stabilizing membrane protein.[22] Results from experiments with gelatin-induced aggregation of rat erythrocytes have indicated that aggregation is an energy requiring process involving a contractile protein situated on the outer surface of the cell membrane.[23] It is claimed that effective NAA bind to the protein and inhibit its adenosine triphosphatase and contractile properties. The normalizing effect[24] of antiinflammatory agents on a serum protein sulfhydryl-disulfide interchange reaction in rats with adjuvant-induced arthritis and the accelerating effect[25] of NAA in an *in vitro* interchange reaction may be relevant to this area.

A striking resemblance is observed between the structures of a large group of compounds active as fibrinolytic agents in *in vitro* screens[26] and the structures of many acidic NAA. Furthermore, the *in vivo* antiinflammatory activity of these NAA correlates with their *in vitro* fibrinolytic activity.[27] It has been shown that rats with adjuvant arthritis had highly increased euglobulin clot lysis times.[28] These times were reduced towards normal by treating the rats with phenylbutazone at a dosage which reduced the size of the secondary lesions. Blood fibrinolytic activity was found to be inversely related to the degree of RA.[29]

Some recent investigations[30-32] have failed to substantiate earlier claims that NAA are effective as a result of their ability to stabilize lysosome membranes. It has been concluded,[32] however, that lysosome membrane stability is very much dependent on experimental conditions and that *in vitro* methods using rat liver lysosomes are not sufficient for studying the effect of antiinflammatory drugs. Increased lysosomal enzyme activity in homogenates of rat paws paralleled increases in paw volume in rats with adjuvant-induced arthritis.[33] Oral administration of phenylbutazone arrested increases in both enzyme activity and paw edema. Above normal lysozyme activity was found in the serum and synovial fluid of a significent proportion of patients with RA.[34]

Miscellaneous observations include the fact that the antiinflammatory effect of NAA in the rat appears to require a normal functioning of the thyroid gland.[35,36] Increased protocollagen proline hydroxylase activity has been noted in rheumatoid synovial tissue.[37] The excretion of hydroxyproline in urine corresponded with the activity and extent of the arthritic process.[38] Antiinflammatory agents reduced the hydroxyproline excretion.

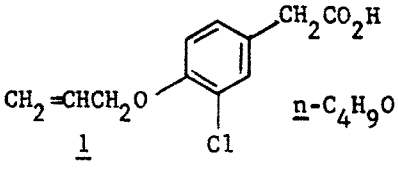
The use of NAA has invariably been accompanied by gastrointestinal irritation. In the rat the operation of the enterohepatic cycle has been shown to be an important factor in ulcer formation due to indomethacin[39,40] and flufenamic acid.[41] Correlations between the antiinflammatory potency of NAA and gastric irritation in the rat have been claimed[42] and denied.[43] Leads to a solution of the irritation problem may have been provided by the observations that spironolactone[44] and ϵ-p-chlorocarbobenzoxy-L-lysin-OMe·HCl[45] prevented the ulcerogenic activity of NAA in rats, with the former causing only a limited depression of antiinflammatory activity.

The common practice of clinically assessing NAA concomitantly with

unrestricted quantities of aspirin has now been questioned. In rat studies, salicylic acid decreased plasma concentrations of indomethacin,[46] and aspirin antagonized expected therapeutic effects of NAA on adjuvant-induced arthritis.[47] More importantly, from a study using aspirin with indomethacin in patients with RA, evidence was presented suggesting impaired gastrointestinal absorption of the indomethacin.[48]

Agents under Investigation *

Arylalkanoic Acids and Related Compounds - The detailed pharmacology of alclofenac (1) has appeared.[49] In osteoarthritis, 1.5 g/day of 1 was found to be equivalent to 0.3 g/day of phenylbutazone.[50] In other degenerative and inflammatory diseases, 3.0 g/day of 1 was found to be equivalent to 75 mg/day of indomethacin.[50] Better tolerance of 1 was reported. Additional evidence has appeared for the clinical efficacy of bufexamac (2) in RA and osteoarthritis.[51-53] Contrary to previous reports, the antiinflammatory (AI) activity of 2 in rats is not related to adrenocortical stimulation.[54] Fenclozic acid (3), which undergoes the unusual NIH shift in the rat and dog,[55] has been withdrawn from clinical study because of hepatotoxicity.[56]



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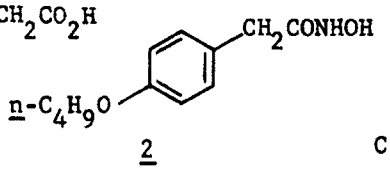

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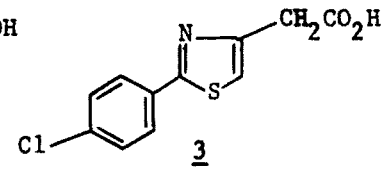

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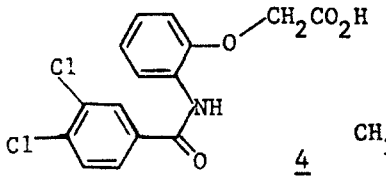
The preliminary pharmacology and structure-activity relationships of a series of relatively non-toxic o-benzamidophenoxy- and o-benzamidophenylalkanoic acids have been published.[57] One of these open-chain indomethacin analogs, clamidoxic acid (4), with AI activity equivalent to phenylbutazone in the carrageenin rat foot edema test (CE), is undergoing clinical evaluation in rheumatic conditions. Naproxen [5, R = CO_2H, (+)-isomer], the most potent compound of a series of 2-naphthylacetic acids,[58,59] had 11 times the AI activity (CE) of phenylbutazone and is now in the clinic. Naproxol (5, R = CH_2OH), the corresponding carbinol, was biologically equivalent.[58] In a double blind study,[60] metiazinic acid (6) was judged superior to placebo in ankylosing spondylitis, RA, and osteoarthritis. 3-[5-



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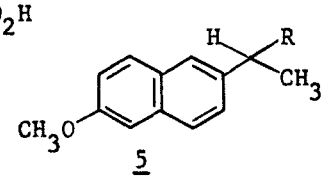

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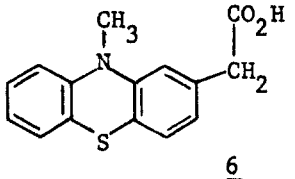

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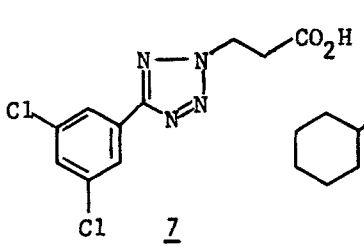
(3,5-Dichlorophenyl)-2-tetrazolyl]propionic acid (7), one of a series of aryltetrazolylalkanoic acids,[61] had 3.6 times the potency of phenylbutazone

* Unless stated otherwise, agents were administered orally.

in the adjuvant arthritis assay. Racemic 8, one of a series of indan-1-carboxylic acids with a conformationally fixed carboxyl group, gave 30% inhibition of edema (CE) at a dose of 3.7 mg/kg.[62] Most of the activity resided in the (-)-isomer. Significant AI activity (CE) has also been found for some p-cyclohexylphenylpropionic acids (9) and related compounds.[63]



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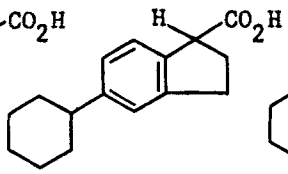

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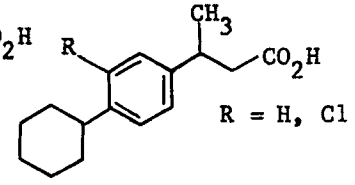
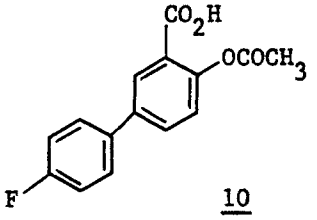


9

Salicylic Acids and Fenamic Acids - Flufenisal (10), an analgetic-antiinflammatory agent, was reported to have 4 times the AI potency of aspirin in acute animal screens, with less gastrointestinal irritation.[64] The aza analog 11 had activity approaching that of 10, whilst 12 was essentially inactive.[65] In a clinical study of its analgetic properties, 10 had 2-4 times the potency of aspirin with almost double the duration of action.[66] Benorylate (13) at 4-6 g/day had activity comparable to that of aspirin at



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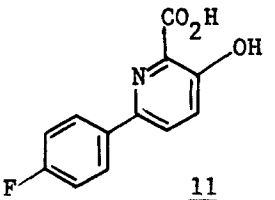

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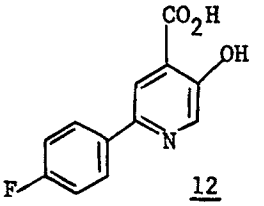
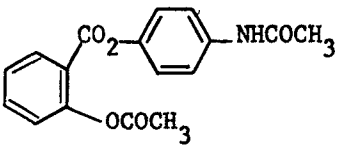


12

2.4-3.6 g/day in chronic rheumatic diseases,[67,68] which is, perhaps, as might be expected from the available salicylate. The fenamic acid analogs 14,[69] and 15,[70] had significant activity in the UV erythema test.



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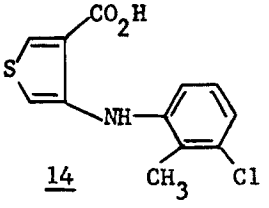

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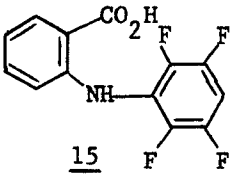
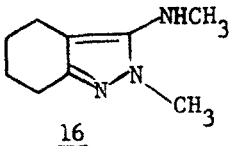
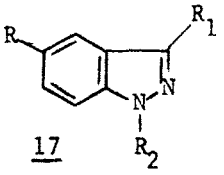


15

Indazoles - Tetrydamine (16) was found to be similar to phenylbutazone in acute and chronic AI screens,[71] superior to aspirin as an analgetic,[71] and devoid of ulcerogenic activity.[72] Extensive animal studies with benzydamine·HCl demonstrated AI but not antiarthritic activity.[73] A series of indazoles for which AI activity was claimed in acute screens is represented by 17a,[74] 17b,[75] 17c,[76] and 17d.[77]



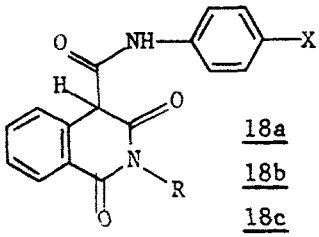
16



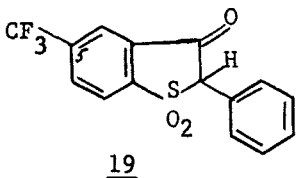
17

	R	R_1	R_2
17a	NH_2	H	C_6H_5CO
17b	H	$O(CH_2)_3N$	p-n-$C_4H_9OCOC_6H_4$
17c	H	$O(CH_2)_2N(CH_3)_2$	m-$CF_3C_6H_4$
17d	H	OH	p-ClC_6H_4

Miscellaneous - Comprehensive studies on some acidic dioxoisoquinoline-4-carboxanilides (18) have been reported.[78] Compounds 18a and 18b were approximately equipotent (CE) to phenylbutazone. Compound 18c is the major human metabolite of 18a. Tesicam (18b) is in the clinic for evaluation in inflammatory diseases.

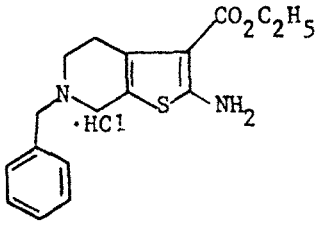


	R	X
18a	CH_3	H
18b	H	Cl
18c	CH_3	OH



19

Compound 19, the most potent member of a series of 2-arylbenzo[b]thiophen-3(2H)-one 1,1-dioxides with both AI and anticoagulant properties,[79] had 1.24 times the activity (CE) of phenylbutazone. All compounds unsubstituted at position 5 had anticoagulant activity.



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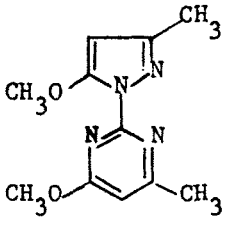
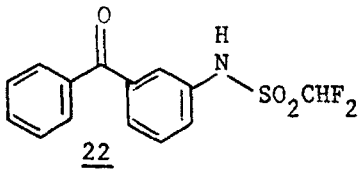
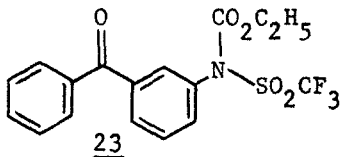


21



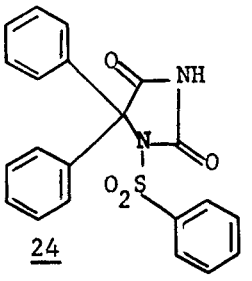
22



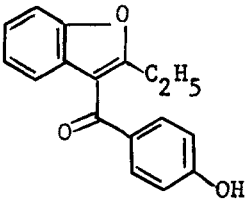
23

The ability to stabilize rat liver lysosomes and inhibit platelet aggregation was reported [80] for the weak antiinflammatory-analgetic thieno[2,3-c]pyridine 20 (Y-3642·HCl).[81] Mepirizole (21) had twice the AI potency (CE) of aminopyrine.[82] Clinical efficacy has been claimed for 21 in a wide range of inflammatory afflictions at doses of 150-450 mg/day.[83] Diflumidone (22) and triflumidate (23) were comparable to phenylbutazone in the adjuvant assay.[84] The hydantoin 24 (PC-796) was effective in a variety of

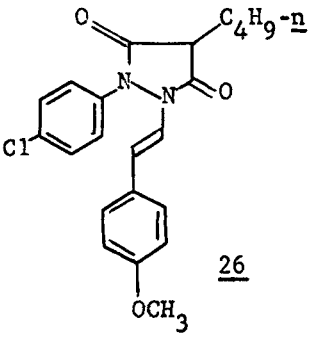
inflammatory models.[85] Benzarone (25), upon parenteral administration, showed AI activity in both acute and chronic tests, and fibrinolytic activity in both in vitro and in vivo/in vitro assays.[86] Some styryl analogs, e.g. 26, of phenylbutazone had comparable activity (CE).[87]



24



25



26

Additional reports of the efficacy of penicillamine in RA have appeared.[88-91] The parenteral use of gold was judged efficacious and safe for chronic polyarthritis when used with frequent laboratory checks.[92] Retikinonase I,[93] a neutral proteinase, and chymostatin,[94] a chymotrypsin inhibitor, both isolated from Streptomyces species, had potent activity (CE, i.p.). Side effects were noted in almost all patients receiving cutaneous treatment with DMSO for humero-scapular periarthrosis, with one patient developing reversible changes in the lens of the eye.[95] Prostaglandin E_2 was active in rat adjuvant arthritis (200-500 μg, s.c., b.i.d.), but inactive in acute screens.[96] Activity (CE) has been claimed for some saponins and other natural products.[97] The AI activity (CE, i.p.) of tocopheronolactone was comparable to that of hydrocortisone acetate.[98] Carrageenin was found to inhibit inflammation in rats[99] and mice,[100] when given i.p. before various irritants, including itself. The AI effect of the carrageenin appears to be due to its ability to deplete kininogen.[101] Tribenoside inhibited the local Shwartzman phenomenon in rabbits.[102] The use of chloroquine in rheumatology has been appraised.[103]

Immunosuppressives - Based on the premise that immunological mechanisms are important in the pathogenesis of some rheumatic diseases, a number of cytotoxic agents have recently received clinical trial in this area. New reports have appeared on azathioprine,[104,105] chlorambucil,[106] cyclophosphamide,[107, 108] and 6-azauridine triacetate.[109] The resulting picture is confusing. Some of the trials have lacked adequate controls, and oftentimes the cytotoxic drugs have been administered together with steroids or aspirin. Some benefit has been observed but often at the high price of severe side effects. Animal studies[110-113] have underlined the importance of the timing of drug administration in autoimmune diseases, with the established disease being less amenable to modification. It has been concluded[114] that azathioprine and chlorambucil fail to suppress immune responses of patients with RA, and that antigen-sensitive and antibody-producing lymphocytes escape inactivation despite a fall in the total number of circulating lymphocytes. Thus the position of the cytotoxic drugs in the treatment of the

rheumatic diseases remains uncertain.

Comment - Although there are several promising agents now under investigation, most bear a close structural resemblance to accepted but inadequate drugs. We feel there is a need for both a better understanding of the inflammatory processes and for new screening methods with greater relevance to the clinical disease picture. By these means, new structural models will surely be discovered which will lead to the eventual attainment of the research goal.

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Chapter 19. Anti-Diabetic Agents

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Introduction - In the 50 years since Banting and Best first isolated insulin from dog pancreas, we have learned much about insulin's synthesis, structure, release and action on numerous tissues. However, we do not yet fully understand the factors controlling these varied aspects in the laboratory animal or man, much less the alterations in diabetes mellitus. Diabetes mellitus in man is broadly classified in two types: the maturity onset type characterized by a relative decrease in functional insulin, and the juvenile type in which there is a complete lack of insulin. Both groups suffer from the acute symptoms of diabetes (elevated fasting glycemia, glycosuria, glucose intolerance and derangements in handling other substrates) in addition to the long term complications (neuropathy, retinopathy, microangiopathy, etc.).

Recent reviews[1-11] have discussed many aspects of insulin synthesis, release and effects on metabolism. It is the purpose of this chapter to highlight more recent advances in the above areas as well as recent concepts regarding the etiology of diabetic complications. In addition we will briefly examine current methods of therapy. Since the major developments in diabetes research have been biological in nature, we have chosen to concentrate our review in this area.

Insulin and Glucagon Homeostasis - Recent studies[12] on the morphology of the beta-cells of the pancreatic Islets of Langerhans (the cells responsible for synthesis, storage and release of insulin) described the functional areas of synthesis and storage of insulin as well as some facets of the release of the stored hormone. Insulin is synthesized on the ribosomes and "packaged" in the Golgi apparatus to produce storage granules. When appropriately stimulated these granules migrate to the outer edge of the beta-cell, possibly via the microtubular system, fuse with the cell membrane and expel their contents extracellularly by emiocytosis.

Biochemical advances in understanding insulin synthesis include the identification and measurement of proinsulin *in vitro* and in man[13-17]. The amino acid sequence of proinsulin has been determined and the amount of the connecting or C peptide can be measured in plasma[18]. The detection of proinsulin in human plasma in addition to its lower biological activity, suggested that a higher proportion of proinsulin might explain the functional lack of insulin in some maturity onset diabetics. This has been shown not to be the case[15,16]. Evidence has also been presented to show that newly synthesized proinsulin is often released in preference to

stored insulin[17]. Present work in this area centers on isolating the enzymes which convert proinsulin to insulin in the beta-cell.

In conjunction with the above, other experiments in man and in perfused rat pancreas indicate that there are two pools of insulin[19-23]. One pool appears to be very labile and readily releasable under various stimulating conditions; the second pool is much larger, includes newly synthesized insulin, and in some manner helps replenish the readily exhausted labile pool of insulin.

A number of factors influencing insulin release have been identified; however, a unifying hypothesis on the signal for insulin release remains difficult to establish. Numerous studies have shown the very close relationship between glucose metabolism by pancreatic islets and the release of insulin[2,5,7,9,24-33]. These include measurement of levels of glucose metabolites (glucose-6-phosphate, 6-phosphogluconate, fructose diphosphate plus trioses), enzyme activities (glucokinase, glucose-6-phosphatase, glucose-6-phosphate dehydrogenase and others) and cofactors (NADP, NADPH). These studies have led to the thesis that some property of the pentose shunt in islets is closely connected to insulin release. Evidence points to NADPH generation as a possible trigger for release. The pentose shunt is very active in islets. NADPH and NADH stimulate *in vitro* release of insulin (fish islets) and NADP and NAD are ineffective[34]. Xylitol, which releases insulin *in vitro* and *in vivo*, can reduce these nucleotides in islet homogenates independent of its metabolism[35,36]. Glucose-stimulated insulin release may also in part be independent of glucose metabolism, suggesting the existence of a glucose-receptor for release[37].

Certain amino acids and protein ingestion cause release of both insulin[2,7,38-43] and glucagon[42,43]. The release of glucagon after protein ingestion seems logical, since without a carbohydrate source, insulin release would lead to hypoglycemia unless glucagon were present to enhance glycogenolysis and gluconeogenesis. In normal man, glucose exerts negative feed back on glucagon release. If blood glucose levels of normal man are elevated to diabetic levels, glucagon levels decrease as insulin levels increase. The absolute levels of glucagon under these conditions are substantially below that found in diabetic man. Diabetics exhibit a relative hyperglucagonemia[44,45], which is unresponsive to elevations of blood glucose, suggesting a significant role for glucagon in the diabetic syndrome.

Studies with 2-aminobicycloheptane-2-carboxylic acid (BCH), a non-metabolizeable amino acid analog, gives support to the concept that amino acids do not have to be metabolized to release insulin[46]. BCH stimulates insulin release *in vitro* and *in vivo* and it has been suggested that it does so by interacting with transport sites. Another agent with structural similarities to arginine, γ-guanidinobutyramide, has been shown to stimulate glucose utilization by muscle and adipose tissue in addition to stimulating insulin release[47,48]. It has also been suggested that this agent stimulates insulin synthesis[48]. Another amino acid analog, guanidinoacetic acid has been shown to enhance insulin release from

the perfused rat pancreas[49].

Other factors known to influence insulin release include the gut hormones: secretin, pancreozymin and gastrin[50-53]. Of these, secretin seems to be most important since at physiological levels it may act as a priming agent to enhance the initial phase of insulin release.

Many ions[2,7,10] have been shown to alter the release of insulin (K^+, Mg^{++}, Ca^{++}, Na^+, Ba^{++} and Li^+). Their effects are believed to be related to electrical potentials across cell membranes[54,55]. Changes in potentials across isolated islets have been measured and found to correlate with the release of insulin.

Cyclic-3',5'-adenosine monophosphate (CAMP) appears important in modulating insulin release[56-58]. CAMP itself stimulates the release of insulin *in vitro*[2,7,10]. It has also been shown that islet CAMP levels can be increased with theophylline or glucagon, both of which enhance *in vivo* and *in vitro* insulin release[2,7,10,56-58]. The mechanism by which CAMP effects insulin release is not known; however, it was suggested that under special conditions this effect might be mediated through its action on the glycogen phosphorylase system, as described for other tissues, thereby giving an intracellular source of glucose-6-phosphate[59]. Theophylline has been shown to enhance the depressed initial phase of insulin release in prediabetic subjects, essentially restoring it to normal[60]. Epinephrine inhibits *in vitro* and *in vivo* insulin release[2,7,10,56] concomitantly depressing the level of islet CAMP[56]. This is believed to be epinephrine's mechanism of inhibition of insulin release since beta receptor stimulation with epinephrine in the presence of an alpha blocker[56] leads to a normal accumulation of islet CAMP and no inhibition of hormone release. Recent work in normal man indicates that the beta agonist, isoproterenol, stimulates release of insulin and alpha agonists inhibit insulin release[2,7,10]. Cholinergic stimulation *in vitro* or *in vivo* has been shown to enhance insulin release[2,7,10]. This suggests a control of insulin levels *via* the sympathetic and parasympathetic systems since both types of nerve endings have been described in the islets.

Rational approaches to diabetic therapy by modulating insulin release, depend on a better understanding of the interrelationships and physiological significance of the above factors in controlling normal release. It may then be possible to discern metabolic or functional differences between normal and diabetic islets. A fundamental problem in achieving such an understanding, has been the fact that no one has yet obtained a pancreatic preparation which allows an examination of the beta-cell function alone. Thus it is not only difficult to determine the metabolic response of beta-cells to various stimuli, but also to determine the relative functional contribution of the other islet cell types.

Insulin and Glucose Homeostasis - Insulin deficiency, clinical and experimental, is in general a catabolic state characterized by an excessive

mobilization of protein and lipid stores, a depressed capacity for carbohydrate assimilation and a depressed ability to limit hepatic glucose production. All of these derangements are thought to contribute to the acute diabetic syndrome (i.e. hyperglycemia, glycosuria). It is apparent that levels of blood glucose reflect a balance between rates of glucose assimilation and hepatic glucose production, with the latter becoming increasingly important in the fine control of glucose homeostasis in the post absorptive and fasting states[61,62]. It is the purpose of this section to briefly review, and direct the reader to current articles regarding abnormalities in glucose assimilation and production in the diabetic, as well as current thoughts regarding insulin mechanisms on glucose homeostasis. Discussion of abnormalities in protein and lipid metabolism will be primarily limited to their effects on glucose homeostasis.

It is well documented that insulin deficiency in laboratory animals and man is, in part, characterized by excessive hepatic glucose output[63-71]. This would appear to be grossly the result of an inability of the liver to maintain glycogen stores accompanied by an increased rate of gluconeogenesis. While increased hepatic levels of CAMP are found to occur in alloxan or antiinsulin serum treated animals[72], which is compatible with increased phosphorylase and decreased glycogen synthetase I activities, the role of insulin *per se* in hepatic glycogen metabolism is uncertain. Thus while insulin and glucose administration are known to cause hepatic glycogen deposition, glucose alone is also very efficient in this respect, and it has been difficult to determine the precise role of insulin[73]. Although insulin alone is known to activate glycogen synthetase activity in diabetic livers[74], it is without effect in livers of normal animals[73]. The effect of insulin on glycogen synthetase activity in diabetics however, does not appear to correlate with depression in CAMP levels[75]. Its effect on synthetase activity may be mediated by an insulin induced stimulation of synthetase D phosphatase activity[76]. The most recent work concerned with the regulation of hepatic gluconeogenesis has implicated variations in substrate supply from peripheral tissues as well as hormonal modulation of hepatic CAMP levels, as physiologically important control mechanisms[77-79]. It is becoming increasingly clear that amino acids represent the most important physiological substrates for hepatic gluconeogenesis in both man and laboratory animals[77,78]. In fasting man, a situation in which insulin levels are low and gluconeogenic rates are high, significant release of amino acids from peripheral muscle occurs. Alanine, however, has been shown to be the principal amino acid released by muscle as well as the principal amino acid extracted by the liver, and of the potential glucogenic amino acids, alanine appears to be the preferred substrate[77,80]. Based on correlations between rates of alanine release from peripheral muscle, blood levels of alanine and rates of gluconeogenesis in fasting man, these studies provide convincing evidence that the rate of alanine delivery to the liver plays an important role in regulating rates of gluconeogenesis. It should be pointed out, however, that although increased mobilization of amino acids occurs in fasting man, blood levels of alanine are observed to decline. This implies that hepatic extraction of alanine proceeds at a more rapid rate than does alanine release. This, in turn, implies that in fasting man, although an

increased delivery of alanine to the liver is taking place, the liver itself has developed an increased total capacity for alanine utilization. A similar situation has recently been reported for diabetic man[81]. While a two to threefold increase of several ketogenic amino acids was found to occur in the plasma of ketoacidotic diabetic patients, a 25-40 percent reduction in the levels of glucogenic amino acids was observed. The mechanisms by which insulin stimulates protein synthesis and reduces amino acid mobilization are poorly understood. It is possible that a fundamental action of insulin on protein metabolism may be the induction of a "translation factor" which in turn allows for polysome formation[82,83]. It is still not clear, however, to what extent an insulin effect at the cell membrane or an insulin effect on amino acid flux across the cell is necessary for observed effects at the nucleic acid level[84,85].

The basis for increased hepatic gluconeogenic capacity in experimental diabetes, has for years been ascribed, in part, to observed increases in the activities of rate limiting gluconeogenic enzymes[86-88]. However, the mechanisms by which insulin, or its deficiency, alter the activity of these enzymes, have not been clear. A great deal of attention over the past few years has been given to the role of CAMP in the regulation of gluconeogenesis, and compelling evidence now exists that alterations in hepatic CAMP levels play an important role in diabetic gluconeogenesis[78,79]. The rapid and direct stimulation of gluconeogenesis by glucagon and catecholamines has been shown to be secondary to hormone induced increases in hepatic CAMP levels. The site along the gluconeogenic pathway effected by these hormones (and CAMP itself) is observed to be between pyruvate and phosphoenolpyruvate, which suggests that one of two rate limiting enzymes for gluconeogenesis (i.e. pyruvate carboxylase, phospho enolpyruvate carboxykinase), or both, are activated directly or indirectly by CAMP. Diabetes produced by alloxan or insulin antiserum results in elevated tissue CAMP levels and increased gluconeogenesis which is also ascribed to increased flux between pyruvate and phosphoenolpyruvate. Insulin has a direct inhibitory effect on hepatic gluconeogenesis and opposes the actions of glucagon, catecholamines and insulin antiserum. These effects appear to be secondary to an insulin mediated lowering of hepatic CAMP levels, but the precise mechanism is not yet understood. In general these studies have demonstrated that at least the acute effects of insulin and insulin deficiency on gluconeogenesis may be secondary to altered CAMP levels. In chronically diabetic animals, however, it has been suggested that alterations in the activity of rate limiting enzymes may also be secondary to alterations in enzyme synthesis. In diabetic man, alterations in the activities of rate limiting gluconeogenic enzymes, similar to those observed in experimental diabetes, have recently been observed[89]. The aforementioned evidence that relative hyperglucagonemia may occur in clinical diabetes[44,45] would, of course, provide a hormonal basis for possible increases in the steady state level of hepatic CAMP in diabetic patients.

While it has been known for years that a relative inability to transport glucose across cell membranes (in muscle and adipose tissue) plays a fundamental role in the inability of the diabetic to utilize

glucose, little has been learned about the mechanism by which insulin facilitates transport. Much of the work in recent years, which relates to this problem, has attempted to define the active site on the insulin molecule itself and to define components of the cell membrane which might represent likely insulin receptor systems.

The early work in this area has been extensively reviewed[90,91,92]. The recent electron density data[93] on the three-dimensional structure of insulin produced some insights on the significance of certain amino acids in the insulin molecule, some of which have been suggested by earlier work[91]. In addition, chemical modifications on the amino acids by formation of derivatives[90,94-98], by removal[99-101] or chemical destruction of amino acids[102], and the synthesis of synthetic A and B chains[103,104], have confirmed the importance of the A1-Gly, the A21-Asp, the A19-Tyr, the A14-Tyr, the B5-His and the three disulfide bridges. The electron density data suggests that some of these are involved in hydrogen bonding to hold the molecule in a unique three-dimensional structure. The A21-Asp, which is essential to activity, is not involved in this type of hydrogen bonding and rests on the exterior surface of the molecule.

A recent attempt to analyze the significant amino acids in insulin and correlate these with the active sites and binding sites has been made[91,105]. In view of the three-dimensional data on insulin, many of the invariant residues of mammalian insulins appear to be on the outside of a common surface, in a predominantly hydrophobic region and become exposed when the dimer dissociates. If this region is important for the attachment of insulin to its receptors, then the mechanism of attachment involves a complementary fit[91]. Modification of the α-amino group of A1-Gly by groups larger than acetyl causes a loss in activity. Since A1-Gly is involved in this hydrophobic sector, and stabilizes the active site probably by hydrogen bonding, modifications lead to drastic distortions of the configuration of the invariant residues. A similar explanation is invoked for the essential amino acid A21-Asp[105].

Previously, indirect evidence suggested that cellular sulfhydryl groups may be involved in the mediation of effects of insulin on target tissue[90]. A number of thiols produced insulin-like activity in isolated fat cells which was not due to non specific transport processes[106]. Also a nucleophilic attack of sodium sulfide on the disulfide bonds of insulin indicated that loss in biological activity paralleled the reactivity of these disulfide bonds[107]. On the other hand, thiol blocking groups such as N-ethylmaleimide do not inhibit the binding of insulin to smooth muscle[108,109], suggesting that a thiol receptor is not involved at the cell surface[108], and there is no data to suggest a correlation between binding to tissue and metabolic effects[110]. The binding of insulin to muscle is believed mediated through the reaction of insulin with tryptophan[111]. In fact the site of action is believed to be a peptide containing tryptophan since 2-hydroxy-5-nitrobenzyl bromide, which reacts selectively with tryptophan, blocks the effects of insulin. Sugar and amino acid transport are stimulated by two different tryptophan recognition sites, neither of which is involved in the insulin stimulation of protein synthesis[112].

It has been suggested that insulin and digestive enzymes are at least ontogenetically related via the digestive tract[113]. Pepsin and pepsinogen behave and produce some metabolic effects similar to insulin in muscle. In rat adipose tissue, oxytocin and some synthetic analogs act like insulin in stimulating the uptake and oxidation of glucose and promoting lipid and protein synthesis although through a different mechanism[114]. It has been shown that chymotrypsin and trypsin at low concentration destroy the binding site of insulin, or the insulin effector system, without destroying insulin or the metabolic integrity of the cell[115]. It was concluded that a trypsin sensitive polypeptide is an essential component of the insulin receptor site of isolated fat cells[115-117].

In order to develop a valid concept on the active site of insulin and thereby construct a synthetic model it would be helpful to have the many known chemical modifications evaluated in those biochemical systems *known* to be affected by insulin. Furthermore these modified insulins should be evaluated in test systems now utilized for defining the tissue receptor site. Finally, by comparing this data with circular dichroism, sedimentation data, and in certain selected cases, electron density data, it should be possible to analyze the significance of insulin's three-dimensional configuration for the significant amino acids relative to the disulfide bridges[90].

While glucose assimilation in diabetes is to a large extent depressed due to altered transport capability, it is also clear that insulin is capable of effecting carbohydrate utilization in peripheral tissues in the absence of stimulating a transport process[118]. In other words, insulin acts not only to facilitate glucose entry but also to influence its intracellular fate. It follows then, that simply accelerating glucose transport in the diabetic would not necessarily guarantee normal intracellular glucose utilization. Effects of insulin on transport-independent glucose disposition in liver, muscle and adipose tissue, and the possible mechanisms involved, can be found in several recent articles[76,118-124].

The influence of increased lipid mobilization in diabetes on glucose production and utilization is controversial. Recent articles concerning the effects of fatty acid metabolism on glucose assimilation, gluconeogenesis and glucose homeostasis in general[125], and a possible role for antilipolytic agents in diabetes[126], have appeared.

Chronic Complications - A great deal of interest has been generated in recent years, regarding the possibility that polyol accumulation may be fundamental to the development of diabetic complications in certain tissues. While sorbitol accumulation in the lens has long been implicated as being causally related to diabetic cataract formation[127-130], more recent work has provided compelling evidence that a similar situation may exist for the development of diabetic neuropathy[131-134] and macroangiopathy[135].

Generally speaking, these studies have demonstrated the presence, in pertinent tissues, of aldose reductase, which catalyzes the NADPH-linked

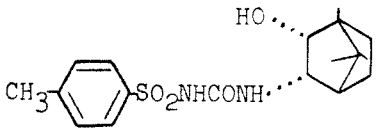
reduction of several sugar aldoses to their respective alcohols. The enzyme has an extremely high k_m for glucose so that maximum rates of sorbitol production depend on high glucose concentrations. Since the tissues involved (lens, peripheral nerves, aorta) are freely permeable to glucose, a hyperglycemic environment allows marked increases in the production of sorbitol, which crosses cell membranes with great difficulty. The resulting increased intracellular level of sorbitol leads to increased osmotic pressure and intracellular edema. It has been strongly suggested that polyol-induced tissue edema may be fundamental to lenticular fiber destruction in cataracts, segmental demyelination in neuropathy and possibly the vascular pathology of macroangiopathy. That hyperglycemia alone, however, may not totally account for increased sorbitol levels in the diabetic, has been suggested in studies of peripheral nerve *in vitro*[136]. For the most part, these studies have been carried out in animal models. However, enough correlation now appears to exist between the experimental model and the clinical diabetic, to allow the suggestion that modulation of tissue polyol levels may represent a rational approach to the treatment of some of the chronic complications of diabetes.

Current Therapy - Sulfonylureas - The sulfonylureas clearly stimulate insulin release from the pancreatic beta-cells, but this immediate and transient action alone does not appear to be responsible for the sustained beneficial effects of the sulfonylureas in diabetic patients[2]. The non-insulin releasing effects of sulfonylureas have been thoroughly reviewed[137,138]. Studies with perfused rat livers indicate that sulfonylureas reduce hepatic uptake of endogenously secreted insulin, thereby potentiating the peripheral action of the hormone[139]. *In vitro* studies of sulfonylureas at physiological concentrations on isolated fat cells indicated potent antilipolytic effects[140], although this has been demonstrated *in vivo* only in laboratory animals[141].

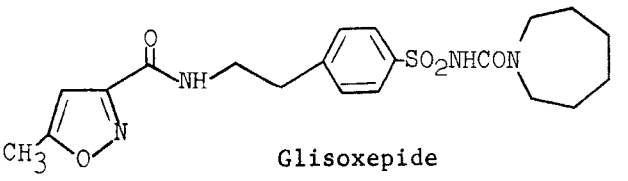
A recent report attempts to dissect the hypoglycemic effects and insulin releasing effects of tolbutamide by comparing the activity of its metabolites hydroxymethyltolbutamide and carboxytolbutamide[142]. Related effects have been seen with corticotrophin A, and its N-acetyl derivative[142,143]. A new concept on the mode of action of sulfonylureas involving a direct suppression of glucagon release was demonstrated *in vitro* and *in vivo* in ducks and man[144].

New High Potency Agents - The most recent additions to the "high potency" sulfonylurea agent glyburide are glybornuride (which is 10-53x more potent than tolbutamide in animals)[145], glisoxepide (with no comparative data)[146], and glydiazinamide (100-500x more potent than tolbutamide)[147]. In view of the large number of patents issued on structurally similar compounds, reports on the activity of other analogs can undoubtedly be expected[147,148].

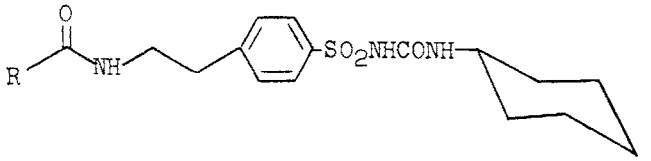
A recent symposium[148] on glyburide thoroughly describes the chemistry, structure-activity parameters, pharmacology, biochemistry, toxicology and clinical findings. A number of clinical studies have shown the successful utilization of the drug[148-150] and the limiting side



Glybornuride



Glisoxepide



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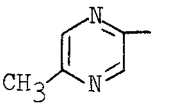

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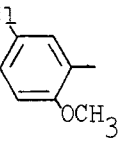

effects[151]. A number of significant in vitro and in vivo (animals) effects of glyburide have been reported [152,153]. Of these, the ability of glyburide to promote insulin release from pancreatic pieces[153] incubated in the absence of glucose might be related to the incidence of hypoglycemic episodes.

Phenformin - The recent status of the mechanism of action of phenformin, has been thoroughly reviewed[154,155]. There have been additional reports on the anorexigenic effect, or lack thereof[156], and on the inhibitory effect of the drug on the rate of intestinal glucose absorption[157]. Although phenformin increases the tolerance to orally administered glucose, it has no effect on intravenous glucose tolerance in humans[158].

UGDP Study - The UGDP has presented its now controversial findings which attempt to compare the long-term effects of certain available methods of treatment for diabetes[159]. Cogent arguments have been published for[159] and against[160] the UGDP conclusions.

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Section V - Topics in Biology

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Chapter 20: Drug Metabolism

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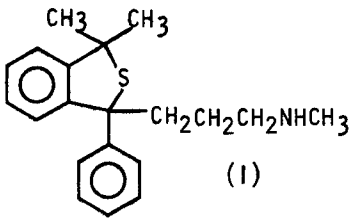
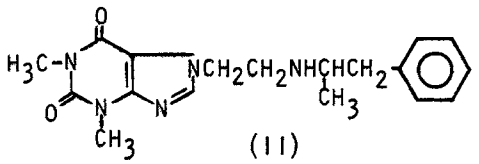
Introduction - Considerable effort was expended in 1970 in comparing, among different species, the biotransformation of a particular compound. More and more, such studies point out the difficulty of finding a suitable animal species for toxicity studies that, qualitatively and quantitatively, produces the metabolites that are excreted by man. In instances, the toxicity of metabolites has been studied; thus, it has been possible to establish unequivocally whether a biotransformation had resulted in a "detoxication." For generic names, structural formulas or chemical names have been provided, if they are not present in The Merck Index.

Psychoactive agents - Seven metabolites of Lu 5-003, a thiophthalane (I) with antidepressive properties, were found in the excreta of rats, dogs, and man[1]. Five of these metabolites, present in all three species, were a result of sulfoxidation, demethylation with subsequent deamination of the side chain, or a combination of these processes. Unaltered (I) and the demethylated metabolite were the major fecal constituents, whereas the sulfoxides and propionic acid derivatives predominated in urine.



(I)

The metabolism in man of fenethylline (II), a psychostimulant related to amphetamine, was studied with the compound tritiated in either the theophylline- or amphetamine-moieties, or with ^{3}H-d-amphetamine itself[2]. Urinary metabolites found after oral dosing with fenethylline labelled in the amphetamine moiety were similar to those obtained after dosing with ^{3}H-d-amphetamine, namely, unchanged amphetamine, hippuric acid, and p-hydroxylated amphetamine. When subjects were given fenethylline labelled in the theophylline moiety, 1,3-dimethyluric acid, 1,3-dimethylxanthine, 1-methyluric acid, and 3-methylxanthine were present in urine. It is suggested that the CNS properties of fenethylline may be a result of its conversion to amphetamine and theophylline.



(II)

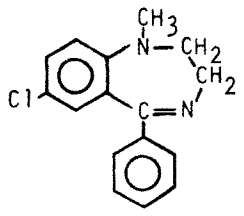
The metabolism of benactyzine was studied in rats[3]. Benactyzine,

benzilic acid (major component), and β-ethylaminoethyl benzilate were excreted in urine, but benzilic acid was also formed nonenzymatically. In another study in rats, the fate of oxypertine, a psychosedative, was examined after i.v. dosing[4]. Little, if any, unaltered oxypertine was present in the urine or bile. The metabolites were the result of O-demethylation of one of the two methoxy groups, alcoholic or aromatic hydroxylations, and the formation of their conjugates. The hydroxylated product of O-demethylation was further oxidized to the -one. Dihydroxylated compounds are also found as major metabolites because, the authors suggest, the monohydroxylated compounds still possess good lipid solubility.

Metabolic alterations of protriptyline were studied in dogs, miniature pigs, and man[5]. In all three species, a primary reaction was oxidation of the cycloheptene moiety, presumably via an epoxide intermediate, yielding, in the urine, mono- and di-hydroxylated metabolites and their conjugates. Another metabolite, identified as 5,10-dihydro-10-formylanthracene-5-propylamine, was thought to be a product of the rearrangement of an hydroxylated intermediate, derived from the metabolically unstable parent epoxide molecule. The primary amine, resulting from N-demethylation, was found only in the dog.

The oxidative metabolism of imipramine was reported in an interesting paper[6]. The authors present evidence from studies with rat liver preparations in vitro that imipramine-N-oxide is not an intermediate in the formation of desmethylimipramine from imipramine. Enzyme activities responsible for demethylation and for the formation of the 2-hydroxylated metabolite could be increased 2-fold by pretreating rats with phenobarbital, whereas the enzyme system responsible for N-oxidation was not induced. Similarly, the demethylation-, but not the N-oxidation-, reaction was sensitive to inhibition by SKF-525A.

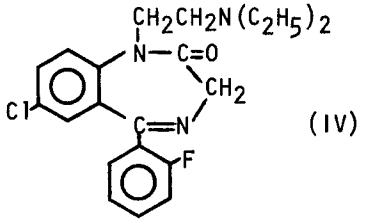
Psychoactive benzodiazepines - The metabolism of prazepam, the cyclopropyl derivative of the N-methyl group of diazepam, was studied in man[7]. This compound is slowly absorbed and slowly excreted, primarily as conjugates. Dealkylated prazepam was the only unconjugated metabolite found in urine, whereas 3-hydroxyprazepam and oxazepam were excreted as conjugates. At least 10 other metabolites were present, but not identified. The principal metabolic reaction for prazepam, but not for diazepam, was 3-hydroxylation. It is suggested that the cyclopropylmethylene group is more resistant to oxidative dealkylation than is a methyl group in the same position.



(III)

Medazepam (III) was metabolized differently by rats, dogs, and man[8]. In rats, but not in dogs or man, the formation of diazepam and its phenolic derivatives was a significant metabolic pathway. Both the dog and man formed oxazepam via a number of metabolic intermediates, but apparently only man was capable of forming

diazepam from medazepam.

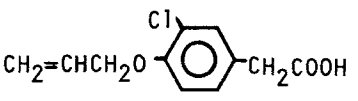


(IV)

The disposition of flurazepam (IV) was examined in a dog and man[9]. The compound was metabolized by successive N-dealkylation to yield, ultimately, the alkylamine, which was excreted in the urine. Man oxidatively deaminated the alkylamine to a conjugate of the homologous alcohol, whereas the dog formed the corresponding carboxylic acid, both presumably via an aldehyde intermediate. A metabolite also found in the urine of both species was the fluorinated analogue of oxazepam, although this metabolite is probably formed by a pathway in which N-dealkylation precedes 3-hydroxylation, since the 3-hydroxy derivative of flurazepam was not detected in urine.

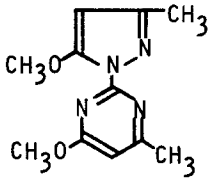
The reduction of nitrazepam in vitro to the 7-amino derivative was studied with preparations of various organs of the rat[10]. The 9000 g fraction of liver was 8 to 9 times more active than that of the kidneys and heart, the next most active tissues. Comparable values for the Km and Vmax for nitrazepam reduction were found with liver preparations of the mouse, rat, guinea pig, and rabbit.

Analgesics - Some of the compounds placed in this category also possess antipyretic or anti-inflammatory activity, or both. The comparative metabolism of 4-allyloxy-3-chlorophenylacetic acid (V) was studied in the rat, rabbit, dog, monkey, and man in a search for an animal species that resembled man in its pattern of excretion and biotransformation[11]. The rat and rabbit excreted (V) differently than did man, and the results of chromatography indicated that different metabolites also were produced by these animal species. Although the dog has many similarities to man in the amounts of free and conjugated compounds present in urine, the excretion of the administered radioactivity differs markedly between these two species. The monkey is closest to man in its mode of excretion and in the similarity of some, but not all, of its metabolites.



Alclofenac (V)

The metabolic transformations of mepirizole (VI) by rats and rabbits were generally similar[12]. The major, inactive urinary metabolite was the carboxylic acid derivative of the methyl group of the pyrazole moiety. This methyl group, as well as the one on the pyrimidine ring, are converted first to hydroxymethyl derivatives, but the methyl group on the pyrazole ring is more susceptible to this biotransformation than is the one on the pyrimidine ring. Other metabolites

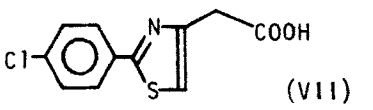


(VI)

identified were the monohydroxylated products of the two unsubstituted carbon atoms of the two rings of mepirizole, and a cleavage product of the molecule, 3-hydroxy-4-methoxy-6-methyl-pyrimidine.

Pentazocine was converted by man to the "trans"-acid and "cis"-alcohol derivatives of the methyl groups of the dimethylallyl side chain[13]. These metabolites and unchanged pentazocine were excreted in the urine. The "trans"-alcohol metabolite, found in the monkey, but not in human urine, is apparently converted rapidly by man to the "trans"-acid metabolite.

The disposition of Myalex (VII) was studied in rats, dogs, and monkeys[14]. The drug is not metabolized by monkeys except for the formation of the acyl glucuronide. Interestingly, the serum and blood of all species contained only unchanged (VII), but the urine of rats and dogs contained metabolites resulting from an "NIH shift" in which hydroxylation at the para position resulted in the loss or ortho migration of the chloro substituent. Glucuronide conjugates of these metabolites are present in the urine of rats and dogs and in rat bile; they are present unconjugated in the feces of either species.



(VII)

Cardiovascular agents - The metabolites of metoclopramide, a compound related to procainamide, were isolated from rabbit urine[15]. They were identified as mono-N-de-ethylated metoclopramide, 4-amino-5-chloro-2-methoxy benzoic acid, an unidentified metabolite that is an oxidation product of the aromatic amino group, and the sulfate and glucuronide conjugates of metoclopramide. Acetylation of the aromatic amino group apparently does not occur in the rabbit.

The metabolism of the selective adrenergic beta-blocking agent, practolol (4-(2-hydroxy-3-isopropylaminopropoxy)acetanilide), was studied in the rat, mouse, and dog[16]. More than 85% of the dose was excreted in the urine as unchanged practolol by the dog and rat. In the rat, 18 metabolites were also excreted, two of them being identified as 2-hydroxy-4-(2-hydroxy-3-isopropylaminopropoxy)acetanilide and its glucuronide conjugate. Deacetylation of practolol appeared to occur to a small extent.

The disposition of nitroglycerin was the subject of two reports. In one of them, rat blood serum was found to contain an organic nitrate reductase that resulted in the reduction of the nitrate group at carbon-2 to nitrite, in preference to those attached to the terminal carbons, and subsequent de-esterification[17]. In the other study, no correlation was found between the absolute blood levels of nitroglycerin and its pharmacological effects[18]. It is suggested that a rapidly changing concentration gradient of nitroglycerin, rather than high blood levels per se, are required to elicit an anti-anginal effect.

The metabolism of pentaerythritol tetranitrate by man resulted

primarily in the formation of pentaerythritol, its mononitrate, and small amounts of the dinitrate[19].

The biotransformation of a racemic mixture of the R- and S-isomers of warfarin was studied in man after oral administration[20]. Urinary metabolites were the result of two types of reactions. One of them involved hydroxylation at either the 6- or 7-position of the coumarin portion, while the other involved a reduction of the acetonyl side chain to yield two diastereoisomeric alcohols.

Biological disposition of stereoisomers - The fates of (±)-, (-)-, and (+)-amphetamine were studied in man, monkeys, dogs, rats, rabbits, mice, and guinea pigs[21]. Man, monkeys, and dogs metabolize the drug similarly; the major transformation products were benzoic acid and its conjugates. Rats, rabbits, mice, and guinea pigs metabolize amphetamine differently from one another and from man, monkeys, and dogs. Owing to the complexity of the study, the original paper should be consulted for additional details.

The conversion of nicotine-1'-oxide and its stereoisomers was studied in animal tissues in vitro and in the urine of cigarette-smoking humans[22]. When nicotine was oxidized by tissue preparations from animals in vitro, both isomers of nicotine-1'-oxide were produced, but the relative amounts of each varied with the species and with the tissue. Liver preparations from guinea pigs formed similar amounts of the d- and l-isomers of nicotine-1'-oxide, whereas preparations of the livers of mice or the lungs of guinea pigs formed more of the l-isomer. Cigarette-smoking humans excrete in their urine predominantly the d-isomer.

Insecticides or their synergists - The metabolism of the bromo derivatives of cyclo-pentane, -hexane, and -heptane were studied in rabbits[23]. The results indicate that all three compounds are hydroxylated at the 2-position, with the trans isomer predominating over the cis.

A study in the rat reinvestigated the nature of the major fecal metabolite of dieldrin[24]. Its structure is thought to be that of dieldrin with an hydroxyl group at position-9. In mice, the toxicity of the metabolite was at least five times less than that of dieldrin.

The biotransformations of some methylenedioxyphenyl insecticide synergists and related compounds were studied in rodents[25]. In mice after oral dosing, the major metabolic pathway for piperonyl butoxide, the diastereoisomers of the n-octylsulfoxides of isosafrole, dihydrosafrole, safrole, and myristicin involves cleavage of the methylenedioxyphenyl moiety, with the loss of the methylene carbon as CO_2. For Tropital (a compound related to piperonyl butoxide), piperonal, piperonyl alcohol, and piperonylic acid, oxidation or conjugation of the side chain, or both, is the major metabolic pathway. After oral doses of piperonyl butoxide, the urine of mice contained compounds that lacked the methylenedioxyphenyl moiety as well as 6-propylpiperonylic acid and its glycine conjugate, whereas, after dosing with Tropital, the urine contained mainly the glycine and glucuronide conjugates of piperonylic acid.

Antineoplastic agents - N-Demethylation was a major metabolic reaction in rats and man in the conversion of 4(5)-(3,3-dimethyl-1-triazeno)-imidazole-5(4)-carboxamide to 4(5)-aminoimidazole-5(4)-carboxamide[26,27].

The transformations of the alkylating agent, methylene dimethanesulphonate (MDS), were studied in rats and mice[28]. Formaldehyde is formed metabolically from MDS and is incorporated into the methyl group of methionine. Other metabolites, derived from formaldehyde, are N-formyl cysteine and N,N'-diformyl cystine. Methanesulphonic acid was identified as a metabolite of MDS in the mouse.

The metabolism of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), a compound active against mouse leukemia L1210, was investigated in several animal species[29]. Generally, very little unchanged CCNU is excreted in urine, the predominant excretory pathway. Metabolites found in the urine of mice and dogs, when ^{14}C was present in the cyclohexyl moiety, were cyclohexylamine, N,N'-dicyclohexylurea, and, presumably, cyclohexyl isocyanate. None of these three metabolites had activity against mouse leukemia L1210.

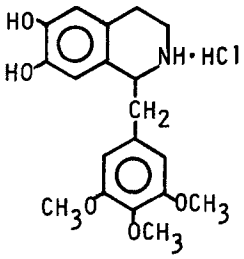
Chemotherapeutic agents - The fate of the aminonucleoside of puromycin (PAN), 6-dimethylamino-9-(3'-amino-3'-deoxy-β-D-ribofuranosyl)purine, was studied either in vitro with rat liver slices or in vivo with the livers of rats that had been dosed i.v. with PAN[30]. Both in vitro and in vivo, PAN was demethylated and phosphorylated to the 5'-nucleotide of 6-methylamino-9-(3'-amino-3'deoxyribofuranosyl)purine. In vitro, an additional metabolite of PAN was formed via a second demethylation to form the nucleoside, 3'-amino-3'-deoxyadenosine.

The fates of cephalexin (CEX), cephaloridine (CER), and 7-(thienyl-2'-acetamido)-3-methylceph-3-em-4-carboxylic acid (TMC) in the alimentary tract of rats were investigated[31]. When the three compounds were given i.p. to rats, they were found to have virtually identical serum half-lives. When the three compounds were injected into the cecum, they were poorly absorbed and were destroyed, probably by the β-lactamases of microorganisms that reside in the cecum; CEX was less susceptible to this inactivation than was CER[32].

Hallucinogenic agents - Several investigators reported the identification and synthesis of active metabolites of $\Delta^1(\Delta^9)$-tetrahydrocannabinol (THC). Incubation of the latter with preparations of rat[33] or rabbit[34] liver, yielded 7(11)-hydroxy-$\Delta^1(\Delta^9)$-THC. Another report described the synthesis of 7-hydroxy-$\Delta^{1(6)}$-THC, an active metabolite, originally isolated from the urine of rabbits[35].

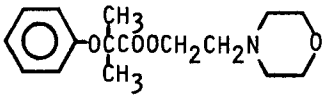
Harmine was O-demethylated by rats[36] and man[37] to harmol and its sulfate and glucuronide conjugates, all of which were excreted in the urine and bile of rats and in the urine of man. Harmine and harmol glucuronide were about equipotent as monoamine oxidase inhibitors, whereas harmol and harmol sulfate were considerably less active in this respect.

Metabolism of other drugs - The metabolism of two bronchodilating agents was studied. One of them, isoprophenamine (1-o-chlorophenyl-2-isopropylaminoethanol), was converted by mice, after oral dosing, to the urinary metabolite, o-chloromandelic acid, whereas rabbits and humans formed o-chlorobenzoic acid and o-chlorohippuric acid[38]. The other bronchodilating agent, trimetoquinol (VIII), was given i.v. to rats and guinea pigs[39]. Biotransformation occurred by O-methylation and subsequent formation of the glucuronide, or by a direct conjugation of glucuronic acid with (VIII). The O-methylation of the d- and l-isomers of (VIII) showed some stereospecificity for the l-isomer, when the reaction was studied in rat and guinea pig liver preparations in vitro.



(VIII)

The antihistaminic, diphenhydramine, was converted by mice, guinea pigs, rabbits, dogs, and monkeys, but not by rats, to diphenylmethoxyacetic acid, the major urinary metabolite[40]. Whereas diphenylmethoxyacetic acid was conjugated by monkeys with glutamine, it was conjugated by dogs with glycine. Morphethylbutyne (IX), an antitussive agent, was administered orally to rats[41]. The compound was rapidly hydrolyzed by tissue and serum esterases to 2-phenoxyisobutyric acid, which was excreted in the urine, feces, or bile. The maximum antitussive activity of morphethylbutyne appears to be at the time when the concentration of 2-phenoxyisobutyric acid is highest in the circulation. The anorexic agent, cloforex, was metabolized by rats after oral dosing or by rat liver preparations in vitro to chlorphentermine (4-chloro-α,α-dimethylphenethylamine), as well as to an apparently conjugated metabolite of the latter[42]. No unchanged cloforex was detected in the urine.

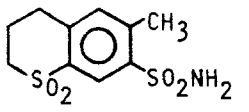


(IX)

The hypoglycemic agent, 2-(p-methoxybenzenesulfonamido)-5-isobutyl-1,3,4-thiadiazole (MIT), has a known history of producing tumors in the bladders of rats, but not of dogs[43]. Three metabolites of MIT were isolated and identified from the urine of rats and dogs after oral dosing[44]. They were 2-(p-hydroxybenzenesulfonamido)-5-isobutyl-1,3,4-thiadiazole and the secondary and tertiary alcohols of the isobutyl side chain of MIT. Two additional metabolites produced only by rats were the alcohol and carboxylic acid produced by hydroxylation and subsequent oxidation of one of the methyl groups of the isobutyl side chain. The metabolites are being evaluated for their carcinogenicity. Neostigmine, the anticholinesterase agent, was converted by rats to the urinary metabolite 3-hydroxyphenyltrimethyl ammonium and its glucuronide[45], as well as to 3-hydroxyphenyldimethylamine, which had been identified as a metabolite in earlier studies[46,47].

The biotransformation of probenecid was studied in man after oral dosing[48]. The metabolites found in the urine are similar to those previously excreted in the bile of rats that had undergone ligation of the renal pedicles[49]; however, β-ether glucuronides of the metabolites are formed by rats, whereas the acyl glucuronides are formed by man.

The fate of the diuretic, meticrane (X), was studied in rats after oral dosing[50]. A portion of the dose was converted to hydroxymeticrane (hydroxylation para to the SO_2), which was excreted unconjugated in the urine and feces and conjugated in the bile. Unchanged metricane was excreted mainly in the urine; in the feces, the amount of meticrane excreted was a function of the amount of the dose administered.



(X)

Microsomes obtained from the livers of rats that had been induced with 3-methylcholanthrene were used to study the metabolism of substituted anisoles and acetanilides in vitro[51]. The substituents studied were F, Cl, Br, I, CH_3, OCH_3, CF_3, and NO_2. It was found that steric factors greatly influenced the metabolites formed from the aromatic substrates. A number of ortho-substituents blocked the normal para-hydroxylation of acetanilides. It is suggested that "steric effects of the ortho-substituent, which may involve the relative configuration of the acetamido group and the aromatic ring, prevent proper binding of acetanilides to the enzyme(s) and thus prevent metabolism by para-attack." Para-hydroxylation of 4-halo-substituted acetanilides resulted in the loss or migration of the halogen. The novel formation of 3-hydroxyanisole from 4-iodoanisole was observed. A similar reaction did not occur with 4-iodoacetanilide.

Studies with microsomal preparations of rat liver in vitro demonstrated that members of a series of α-thiocarboxylic acids were converted to the corresponding sulfoxides[52].

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Chapter 21. Biological Actions of Cyclic AMP Analogs

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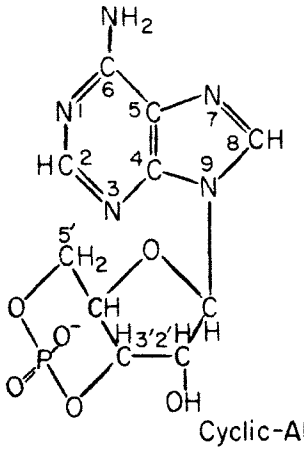
Adenosine 3',5'-cyclic phosphate (cAMP) has been established as an intracellular "second messenger" mediating the action of a number of hormones. According to this concept, the hormone (the first messenger) interacts at the membrane of the target cell to stimulate the intracellular formation of a second messenger which then facilitates the physiological or metabolic processes within the cell. This general concept, the growing list of hormones which affect intracellular levels of cAMP, and the amazing diversity of cellular processes known to be influenced by the cyclic nucleotide have been reviewed by Sutherland and his associates[1,2]. A number of hormones (especially amine and peptide hormones) increase intracellular levels of cAMP by stimulating adenyl cyclase, the enzyme which catalyzes the formation of cAMP from ATP and which resides primarily in the plasma membrane of most cells. The cyclic nucleotide is degraded to 5'-adenylic acid by ribonucleoside 3',5'-cyclic phosphate diesterase, and so a hormone or drug may alter intracellular levels of cAMP by an action (positive or negative) on this enzyme as well. Several cellular processes affected by cAMP such as glycogenolysis, glycolysis, gluconeogenesis, glycogen synthesis, lipolysis, ketogenesis, etc., appear to be due to the action of the cyclic nucleotide on specific enzymes or enzyme systems. The involvement of cAMP in other diverse processes such as inotropism, permeability changes, hormone release, melanocyte dispersion, platelet aggregation, steroidogenesis, neuromuscular transmission and enzyme induction is less well understood.

The role of cAMP as a "second messenger" implies that the compound should mimic the action of a hormone when administered exogenously to the target tissue. There are however, relatively few instances where this has been demonstrated; the cyclic nucleotide is generally quite inert when added to isolated tissues or when administered intravenously to intact animals, even in doses several orders of magnitude greater than that which exists physiologically (10^{-8} to 10^{-6} M). One reason for this is the very low permeability of cAMP across cell membranes, presumably a reflection of the net negative charge on the molecule at physiological pH. Furthermore, any cAMP which may cross membrane barriers could be rapidly destroyed by cyclic phosphate diesterase. The need has arisen for chemically related compounds which might possess improved permeability characteristics and which might resist enzymic destruction. Following the availability of general procedures for the synthesis of both ribo- and deoxyribonucleoside 3',5'-cyclic phosphates[3,4] a variety of derivatives and analogs have become available. In general, these compounds involve substitution in the 6-, 7-, or 8- positions of the purine ring, alteration of the cyclic phosphate-diester group, or acylation of the amino group in the N^6- position or of the 2'-hydroxyl of the ribose moiety. Use of these materials has provided valuable information regarding the validity of the second messenger

concept; they have contributed to an understanding of structure-activity relationships and to the specificity and mechanism of action of cAMP in a number of enzyme systems and to some understanding of the structural requirements for cyclic phosphate diesterase action. Compounds of this type with some level of organ or tissue specificity carry the potential for the development of useful therapeutic agents. This essay attempts to review some of those studies concerned with the actions of various analogs on enzymes and physiological systems in which cAMP has been implicated as a mediator of hormone action.


Cyclic-AMP

Hepatic Glucose Formation - The first chemical modification of cAMP involved acylation at the 6-amino and 2'-hydroxyl positions yielding the N^6-monooctanoyl, N^6-monobutyryl, N^6,2'-O-dibutyryl, 2'-O-monobutyryl and 2'-O-monooctanoyl derivatives[5]. Of these, the N^6,2'-O-dibutyryl analog (DBcAMP) has been particularily useful and generally mimics hormone action in many systems. Each of the above compounds was more effective than cAMP in producing hyperglycemia in dogs following intravenous administration (13 μmoles/Kg) and the duration of the response was also longer[5,6]. The dibutyryl derivative was also more effective than cAMP in producing hyperglycemia when infused intravenously (0.2 mg/Kg/min for 1 to 1 1/2 hrs) into human volunteers[7]. The glycogenolytic activity of various derivatives has been studied in more detail using isolated perfused livers from fed rats. Infusions of 2'-O-monobutyryl-cAMP (7.8 mmoles/hr) into isolated rat livers resulted in greater increases in glucose output than equimolar amounts of cAMP[8] and liver glycogen content was also more extensively reduced. DBcAMP was found to promote greater glucose output than the 2'-O-butyryl derivative when infused at the same dose[9]. The dibutyryl derivative was especially potent early in the perfusion (during the first 30 min), supporting the possibility that it penetrated the liver cell even more rapidly than the monobutyryl derivative or was less susceptible to destruction. In similar studies, tubercidin 3',5'-cyclic phosphate (cTuMP) (7-deaza-adenosine 3',5'-cyclic phosphate) was found to be as potent as DBcAMP in stimulating glucose output[10]. Inosine 3',5'-cyclic phosphate (cIMP)[10,11] and its 2'-O-monobutyryl derivative[10] were slightly less potent than the above 2 compounds. The 3',5'-cyclic phosphates of thymidylic, uridylic and cytidylic acids (cTMP, cUMP and cCMP respectively) were not as effective[10,11]. Guanosine 3',5'-cyclic phosphate (cGMP) has been reported to be either relatively ineffective[10] or highly effective[11,12] in stimulating glucose output in perfused livers. These studies seem to indicate that several ribonucleoside 3',5'-cyclic phosphates, particularly DBcAMP, cIMP, the 2'-O-butyryl derivatives of cAMP and cIMP, and cTuMP, display a glucagon-like action on glycogenolysis in liver tissue.

Perfusion of livers from fasted animals provides a means for studying the contribution of gluconeogenesis to hepatic glucose output. cAMP is known to stimulate glucose formation when added to the perfusion fluid of such preparations[13,14]. Menahan and Wieland[15] found that DBcAMP at 10^{-4} or 10^{-5} M stimulated endogenous glucose formation in perfused livers from 24-hour fasted rats to a degree quite like that produced by glucagon. The cyclic purine nucleotides cAMP, cGMP, cIMP also increased gluconeogenesis to the same extent as glucagon[11] although they were considerably less potent than DBcAMP, requiring a perfusion concentration of 1 x 10^{-3} M whereas the dibutyryl analog produced maximal effects at 1 x 10^{-6} M. cCMP and cUMP were less effective at 1 x 10^{-3} M and cTMP was inactive[11]. Stimulation of gluconeogenesis in rat renal cortical slices by cAMP and cIMP has also been demonstrated[16,17]. In this preparation cGMP inhibited gluconeogenesis. DBcAMP also increased endogenous urea formation[12,15,18], ketone body production[15,19] and amino acid uptake[18] by the perfused liver. All these observations add support to the possibility that cAMP mediates the effects of glucagon on gluconeogenesis, ketogenesis, ureogenesis and amino acid uptake by the liver.

Lipolysis in Adipose Tissue - It now appears reasonably well established that hormone-induced lipolysis in adipose tissue is mediated, at least in part, by cAMP. Attempts to induce lipolysis by adding the cyclic nucleotide directly to intact adipose tissue preparations, however, have not generally been successful except in a medium devoid of both Ca^{2+} and Mg^{2+}[20]. This procedure presumably increases the penetration of the cyclic nucleotide sufficient to stimulate the lipolytic process. A large amount of evidence shows that DBcAMP has a marked lipolytic action in isolated epididymal fat pad preparations from rats[21,22] and in isolated fat cells (adipocytes) prepared from rat adipose tissue[21,23-27] and from human adipose tissue[28]. N^6-monobutyryl-cAMP is also lipolytic in rat fat cells[26]. In Krebs-Ringer phosphate medium devoid of both Ca^{2+} and Mg^{2+}, a variety of unsubstituted cyclic nucleotides can apparently penetrate isolated adipocytes and have been examined for lipolytic activity. Under these circumstances DBcAMP and cAMP were now almost equieffective[26,30]. cGMP, dibutyryl-cGMP, cCMP, cIMP and cUMP also stimulated lipolysis but all were either less than one-half as effective as cAMP[30], or required greater concentrations than cAMP or DBcAMP[26]. No activity was observed with cTMP or with deoxyribonucleoside 3',5'-cyclic phosphates except for deoxy-cAMP which was approximately one-third as active as cAMP[26,30].

Some divergence exists between the actions of cAMP, DBcAMP and lipolytic hormones with respect to glucose utilization and lipolysis by fat cells. Lipolytic hormones (epinephrine, glucagon and ACTH) increase glucose utilization and stimulate glucose oxidation[31]. However only low concentrations of DBcAMP (.03-.15 x 10^{-3} M) stimulated glucose oxidation when lipolysis was not stimulated[32], whereas concentrations of DBcAMP (0.3-3 x 10^{-3} M) which stimulated lipolysis actually inhibited glucose oxidation[32,34]. In contrast to DBcAMP and cGMP, cAMP, cCMP, cTMP and cUMP were reported to stimulate glucose utilization[29,34]. However, it has been clearly shown that increased glucose utilization by hormones can be dissociated from their effects on lipolysis[31] and therefore the significance

of these observations are difficult to evaluate.

Adrenal Steroidogenesis - Because ACTH stimulates adrenal cAMP formation and because the cyclic nucleotide is capable of stimulating steroidogenesis, it has been considered that cAMP functions as an intracellular mediator of ACTH action. In accord with this, the intravenous infusion of cAMP, DBcAMP and 2'-O-acetyl-cAMP (20 mg doses) caused significant increases in adrenal corticosterone secretion in hypophysectomized rats[35]. The increases in corticosterone content of adrenal venous blood was greatest with DBcAMP (20 mg was almost as effective as 1 mU of ACTH), intermediate with cAMP, and least with the monoacetyl ester. Stimulation of steroidogenesis by cyclic nucleotides has also been demonstrated using adrenal slices. Corticosterone production was stimulated by cGMP, cUMP, cCMP, and cIMP to approximately the same extent as by cAMP[11,36]; deoxy-cAMP was also active. Deoxy-cTMP and several nucleoside 2',3'-cyclic phosphates were inactive[36]. DBcAMP produced maximal steroidogenesis at 1×10^{-4} M whereas cAMP required concentrations above 1×10^{-3} M[11]. DBcAMP has been reported to be less effective than cAMP (each tested at 1×10^{-3} M) in stimulating steroidogenesis in adrenal cells in tissue culture[37]. However in suspensions of isolated adrenal cells, stimulation of steroidogenesis was much more sensitive to DBcAMP than to cAMP[38]. In this preparation DBcAMP at 1×10^{-5} M produced maximal stimulation (equivalent to 0.1 mU of ACTH) and was 50 times more potent than the parent compound, cAMP.

Secretion of Hormones - Considerable evidence exists that cAMP may be involved in the secretion of protein hormones. A number of analogs have been used to provide further evidence for this possibility. Several N^6-alkyl derivatives have been observed to stimulate the release of hormones from the rat anterior pituitary[39-42]. N^6-monomethyl-cAMP, N^6-dimethyl-cAMP, N^6-n-butyl-cAMP and DBcAMP were more active than cAMP in promoting release of growth hormone (GH) and thyroid stimulating hormone (TSH)[40-42]. The N^6-tert-butyl derivative was less active in stimulating GH release[42] and inactive in stimulating TSH release[40]. Iso-cAMP (in which the ribose moiety is attached to the 3-position of the purine ring) was found to be more active than cAMP in stimulating TSH release[39,40] but less active in stimulating GH release[42]. The concentrations of all these derivatives required to promote hormone release in isolated adenohypophyses was usually 0.5-5 $\times 10^{-3}$ M. None of these derivatives were active in promoting the release of prolactin[41]; iso-cAMP was in fact slightly inhibitory[42]. In another study[43] DBcAMP (5×10^{-3} M) caused a rise in TSH release from rat adenohypophyses *in vitro*. cAMP in concentrations as high as 1×10^{-2} M had no effect. The stimulatory action of DBcAMP was inhibited by L-thyroxine[42]. DBcAMP (1×10^{-3} M) was also found to increase luteinizing hormone (LH) release by rat anterior pituitary *in vitro*[44]. Theophylline (which inhibits 3',5'-cyclic phosphate diesterase) at 10^{-2} M also had a stimulatory effect. When DBcAMP and theophylline were added concomitantly, the response was synergistic. The dibutyryl derivative of cAMP has been found to mimic the actions of TSH with respect to thyroid hormone release in thyroid slices and intact thyroid cells[45-47]. It also mimics TSH with regard to other aspects of thyroid metabolism such as intracellular colloid droplet formation[48,49], iodide uptake[50-52], iodoprotein formation[50,52,53], glucose oxidation[54-56] and the synthesis of RNA

and phospholipids[51,55,57].

Cardiac Inotropism - It is now firmly established that β-adrenergic amines stimulate a rapid rise in cAMP levels in isolated perfused mammalian hearts and facilitate glycogenolysis in this tissue. The increase in cAMP levels precedes the positive inotropic and chronotropic effects. For this, and other reasons, it has been attractive to consider the possibility that cAMP initiates or mediates the effects of adrenergic amines on the mechanical performance of the heart. One difficulty in proving this hypothesis has been the failure to reproduce the inotropic effects of catecholamines with exogenous cAMP[6,58,59]. In addition, early reports[58] indicated that DBcAMP, N^6-monobutyryl-cAMP and N^6-benzoyl-cAMP at concentrations as high as 1×10^{-3} M had no inotropic action on the isolated rat heart. Skelton, Levey and Epstein[60] have clearly demonstrated that DBcAMP has an inotropic effect on isolated cat papillary muscle stimulated electrically. The concentration-dependent increase in isometric tension and rate of tension development at peak concentration (3×10^{-3} M) were similar to those found at peak norepinephrine concentration (10^{-5} M). The dibutyryl analog also caused a marked shift of the force-velocity curve upward and to the right. The contractile response to DBcAMP was not altered by propranolol. Kukovetz and Pöch[61] have shown that DBcAMP and dihexanoyl-cAMP in high doses (10 μmoles) produced strong and long lasting increases in the rate and amplitude of isotonic contractions and increased coronary flow in hearts of guinea pigs, rats and rabbits perfused by the Langendorf technique. The positive inotropic and chronotropic effects did not occur immediately but only 3 to 5 minutes after injection of the cyclic nucleotides. It has also been observed that intravenous administration of DBcAMP to human volunteers resulted in an increase in heart rate[7]. It thus seems highly probable that cAMP is the cellular mediator of the cardio-stimulatory action of adrenergic amines and other substances such as glucagon which stimulate its formation in the heart. Other evidence supporting this possibility has recently been presented[62].

Specific Enzyme Systems - As mentioned earlier several acyl derivatives of cAMP and other 3',5'-cyclic nucleotides were capable of stimulating glucose output in intact dogs[5,6] and in isolated perfused livers[8,12]. Such effects, at least in part, result from the activation of glycogen phosphorylase. In equimolar doses (10^{-5} M), several N^6-acyl and 2'-O-acyl derivatives were more effective than cAMP in activating phosphorylase in dog liver slices[6]. In particular, N^6-monobutyryl-cAMP, DBcAMP and N^6-adamantyl-cAMP were 50-fold more potent than the parent compound; the 2'-O-monobutyryl and 2'-O-monooctanoyl derivatives were less active but still more potent than cAMP. In isolated perfused livers DBcAMP, cAMP and cGMP activated phosphorylase to the same extent as maximal doses of glucagon[12]; DBcAMP was the most potent on a molar basis, 1×10^{-5} M afforded maximal activation. DBcAMP and N^6,2'-O-dihexanoyl-cAMP have been reported to illicit a modest activation of phosphorylase in isolated perfused guinea pig hearts concomitant with an increase in contractility[61]. Quite different relative activities however were obtained when several acyl derivatives were examined for their ability to activate phosphorylase in tissue extracts (liver and heart)[5]. In these preparations the N^6-

monooctanoyl, N^6-monobutyryl and N^6-monoacetyl derivatives were less active than cAMP; DBcAMP was only 2% and 0.4% as active in liver and heart respectively, as the parent compound. 2'-O-acyl derivatives also possessed *very low* activity[5]. The decrease in hepatic glycogen content following perfusion with analogs of cAMP[8,9,12] is due, in part, to phosphorylase activation with facilitated glycogenolysis. Under these circumstances decreased glycogen synthesis resulting from inactivation of glycogen synthetase could also be contributory. It is well established that cAMP facilitates inactivation of glycogen synthetase (synthetase I to D conversion). It might be expected that analogs of cAMP may act in tissues to facilitate the conversion of this enzyme to the less active form. This indeed has been demonstrated. In perfused rat liver DBcAMP (1×10^{-5} M) was about 10 times more potent than cAMP and cGMP in decreasing glycogen synthetase activity[12]. In another study[63] cIMP, cGMP, cCMP and cUMP were as effective as cAMP (all present in perfusate at 1×10^{-3} M). However when these cyclic nucleotides were tested for their ability to activate glycogen synthetase I kinase (the enzyme which catalyzes the conversion of synthetase I to the D form) in rat liver homogenates, cAMP was much more potent, the concentrations for half-maximal conversion (K_a) being approximately 0.3, 3.0, 30, 35, and 100 $\times 10^{-6}$ M for cAMP, cIMP, cCMP, cGMP, cUMP respectively[63]. Likewise the K_a for cAMP, cGMP, cCMP, cUMP and cTMP for the purified rabbit muscle synthetase I kinase was 0.067, 9.9, 8.9, 6.5 and 1300 $\times 10^{-6}$ M respectively[64].

It is now firmly established that cAMP facilitates phosphorylase activation by an interaction with a cAMP-dependent protein kinase[65] which then catalyzes the conversion of nonactive phosphorylase kinase to an activated form. The latter enzyme then catalyzes phosphorylase *b* to *a* conversion (phosphorylase activation). In this system the action of protein kinase is really that of phosphorylase *b* kinase kinase. Since it is not specific for phosphorylase kinase but phosphorylates other proteins as well, it has been given the general name protein kinase[65]. The ability to facilitate conversion of nonactivated phosphorylase kinase to the activated form even with highly purified preparations results from the presence of contaminating amounts of protein kinase, which is the absolute receptor *for* cAMP. Activation of phosphorylase kinase[66] and stimulation of protein kinase with cAMP [65] are both easily measurable and have been extensively studied with highly purified enzymes. As a result, stimulation of glycogenolysis is the most decisively delineated function of cAMP to date. Numerous analogs of the cyclic nucleotide have been examined for their ability to activate phosphorylase kinase from both liver and muscle, and, more directly, to stimulate protein kinase. In early studies, activation of purified cardiac phosphorylase kinase appeared to be quite specific for cAMP (half maximal activation was produced by 5×10^{-8} M)[67]. Ribonucleoside 3',5'-cyclic phosphates of uridine, cytidine, guanosine, deoxyadenosine, deoxycytidine, thymidine and deoxyguanosine were all inactive at 10^{-5} M. When present at 1×10^{-4} M, cUMP, cCMP, cGMP and deoxy-cAMP produced activation equivalent to 100, 80, 75 and 40 per cent respectively of that produced by cAMP at 10^{-7} M[67]. Drummond and Powell[68] have examined the activity of several analogs on activation of the purified rabbit muscle phosphorylase kinase system. The concentration of cAMP

required for half-maximal activation was 7.3×10^{-8} M. cTuMP was slightly more active than cAMP. DBcAMP, cUMP and cCMP were about 1% as active as cAMP. Compounds which involve structural or spatial orientation of the cyclic phosphate diester linkage showed markedly decreased activity. Thus, adenosine 3',5'-cyclic phosphorothioate (>P=S replacing >P=O) was only 0.36% as active as cAMP and adenine xylofuranosyl 3',5'-cyclic phosphate (in which the position of the hydroxyl in the 3'-position is in the opposite plane from cAMP) was inactive at concentrations as high as 6×10^{-4} M. Similarily, a 3'-methylene cyclic phosphonate derivative (differing from cAMP in that a methylene group replaces the hydroxyl in the 3'-position) was inactive at concentrations as high as 4×10^{-4} M. The analogous 5'-methylene cyclic phosphonate compound (a methylene group replacing the 5'-hydroxyl) was about 1% as active as cAMP. These studies point to a rather marked selectivity for an unmodified cyclic phosphate grouping. Kuo and Greengard[69] have examined the effects of several of these analogs more directly on cAMP-dependent protein kinase purified from bovine brain[70] and adipose tissue[71] and on a cGMP-dependent protein kinase which they have isolated from lobster muscle[72]. cTuMP was able to maximally stimulate both the cAMP-dependent and cGMP-dependent protein kinases 69. The concentration of cTuMP required for half-maximal stimulation (K_a) was equal to that of cAMP (1×10^{-7} M) with the enzyme from either brain, heart or adipocytes in agreement with previous findings using the skeletal muscle phosphorylase kinase assay[68]. The K_a for cTuMP (8×10^{-7} M) was greater than that for cGMP (7×10^{-8} M) for the cGMP-dependent protein kinase but less than that for cAMP (3×10^{-6} M) with this enzyme. The 5'-methylene cyclic phosphonate analog had little or no activity on protein kinase[69] in agreement with the phosphorylase kinase system[68]; the 3'-methylene cyclic phosphonate was completely inactive[69]. Recently Du Plooy et al.[73] have studied the effects of a large number of analogs involving primarily substitutions in the 6- and 8-positions of the purine ring using purified phosphorylase kinase preparations from liver and skeletal muscle. Analogs involving substitution at the 6-position of cyclic purine riboside monophosphate (cPuMP) (6-methoxy-; 6-alkylamino-; 6-(1-pentylamino)-; 6-(1-phenylethylamino)-; 6-benzylamino)-; 6-(2'-methylbenzylamino)-; 6-(4'-methylbenzylamino)-; 6-morpholino-; and 6-piperidino-cPuMP) were equally or more potent than cAMP in stimulating the enzyme from both liver and muscle. Similar alterations in the 8-position of cAMP (8-bromo-; 8-allylamino-; 8-benzylamino-; 8-(2'-chlorobenzylamino)-; 8-(4'-methylbenzylamino)-; 8-morpholino-; 8-piperidino-cAMP) and the corresponding derivatives of cIMP were as active or slightly less active than the parent compound in each case[73]. These, and studies previously described[68] would indicate that substitution in the purine ring is less likely to affect specificity and binding to the enzyme than is alteration of the cyclic phosphate diester group.

Catabolism of Analogs - An assumption frequently made when using acyl derivatives of cAMP has been that they are converted by enzymes present in tissues and crude extracts to the parent compound which would then be hydrolyzed by cyclic phosphate diesterase. For example, in order to explain the discrepancies between the activity of acyl derivatives in intact animals and tissue extracts, it was proposed that the acyl compounds

were deacylated by soluble esterases before becoming active[5,6]. Existence of an esterase in liver was proposed as the reason for diacyl and 2'-O-monoacyl derivatives being more active in liver extracts than in extracts from heart[5]. However when ^{3}H-DBcAMP was incubated with intact adipocytes [26,74] only a slight non-enzymic release of ^{3}H-butyric acid could be accounted for, indicating a remarkable stability of this compound in at least one tissue. It is relevant that deacylation of DBcAMP has been reported to occur rather rapidly in aqueous buffer, particularly Krebs-Ringer bicarbonate[75]. When ^{3}H-N^{6}-monobutyryl-cAMP or ^{3}H-DBcAMP were incubated with extracts of rat liver, thyroid or adipose tissue, deacylation did occur[26,56], the highest activity occurring in the cytosol fraction[26]. It was suggested that deacylase activity exists at least for the amide substituent; hydrolysis of the 2'-O-acyl substituent was not studied[26]. The resistance to deacylation exhibited by intact adipocytes[26,74] is not completely explainable. The need for further studies to delineate the catabolism of these and other cAMP analogs in a variety of tissues is obvious.

Several analogs have been examined as substrates for nucleoside cyclic phosphate diesterase purified from a variety of tissues. In particular, N^{6}-monobutyryl-cAMP and DBcAMP are not hydrolyzed by cyclic nucleotide phosphodiesterase from heart[76], liver[10,77], adipose tissue[23,26,78] or brain [68]. Moreover DBcAMP did not inhibit the hydrolysis of cAMP by heart phosphodiesterase[76], indicating a failure to bind to the enzyme. Since DBcAMP is not degraded by the diesterase its biological activity should not be influenced by those materials which either inhibit (theophylline) or stimulate (insulin, nicotinic acid, imidazole) the enzyme. The effect of these compounds on lipolysis produced by DBcAMP has been studied with somewhat conflicting results. Theophylline was found to potentiate DBcAMP-induced lipolysis[24,79] and nicotinic acid inhibited DBcAMP-induced lipolysis[27,79]. Insulin and imidazole have been reported to have either no effect or to inhibit lipolysis induced by DBcAMP[23,24,34]. These results are difficult to interpret at this time.

Several studies have provided some information regarding the hydrolysis of other cyclic nucleotides by cyclic phosphate diesterase. The enzyme from brain hydrolyzes cyclic 3',5'-monophosphates bearing purine bases somewhat more readily than those bearing pyrimidines[4,80]. cCMP was not attacked. Deoxyribonucleoside 3',5'-cyclic phosphates were hydrolyzed at rates slightly less than the corresponding ribonucleoside derivatives[4]. Similar specificity is shown with the dog heart enzyme[81], liver enzyme[77], the enzyme from S. marcescens[82] and from frog erythrocytes[83,84]. cTuMP was hydrolyzed three times more rapidly than cAMP by the rabbit brain enzyme[68]. Adenosine 3',5'-cyclic phosphorothioate and the 3'-methylene cyclic phosphonate analog were neither substrates nor effective inhibitors; the 5'-methylene cyclic phosphonate compound and adenine xylofuranosyl 3', 5'-cyclic phosphate were extremely poor substrates[68]. All these studies indicate that the enzyme displays rather broad specificity with regard to the base moiety; binding is critically dependent on an intact cyclic phosphate diester group and to a lesser extent on an intact sugar moiety. Recently evidence has accumulated that more than one cyclic phosphate

diesterase is present in tissues[85-88]. Their precise substrate requirements and their physiological relevance must await further study.

Very little information is available regarding the mechanisms by which other cyclic nucleotides and derivatives mimic the action of cAMP. The relative potencies of several cyclic nucleotides as lipolytic agents [26,30] were not related to their relative rates of hydrolysis by adipose tissue phosphodiesterase[26,79]. There is no evidence that activity of cyclic purine or pyrimidine nucleotides is secondary to interconversion to cAMP[79]. It has been observed that cGMP and cIMP inhibit the hydrolysis of cAMP by cyclic nucleotide phosphodiesterase from frog erythrocytes[83,84] and that cGMP caused accumulation of cAMP in fat cells[89]. However phosphodiesterase from adipose tissue and liver were not inhibited by cGMP, cIMP, cUMP or cTMP[30,79] and perfusions with cGMP had no effect on hepatic cAMP levels[12]. It would seem that the biological activity of various acyl derivatives may in part be due to resistance to degradation by diesterase before deacylation to the active compound. In addition it is highly probable that cyclic nucleotide analogs with purine or pyrimidine bases may exhibit biological activity directly, reflecting lack of absolute specificity by the receptor. Analogs with better specificity are likely to become of considerable importance to a final understanding of the myriad physiological actions of cAMP. The development of specific analogs as potentially useful pharmacological agents represents an exciting and challenging problem for future research.

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Chapter 22. The Structure-Activity Relationships of Adrenergic Compounds that act on Adenyl Cyclase of the Frog Erythrocyte

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Cyclic 3', 5'-AMP (cyclic AMP) is an intracellular mediator of the actions of a variety of humoral agents including most peptide hormones and the catecholamines.[1] Catecholamines stimulate adenyl cyclase activity in many tissues including liver, brain, skeletal muscle, smooth muscle, cardiac muscle, adipose tissue, lung, spleen and nucleated erythrocytes and many of the metabolic and physiological responses of these tissues to catecholamines can be mimicked by the addition of either exogenous cyclic AMP or its acyl derivative, N^6, $O^{2'}$-dibutyryl cyclic AMP.[2] Since the earliest detectable biochemical effect of the catecholamines appears to be on the activity of membrane-bound adenyl cyclases, it was proposed[2] that the adrenergic receptor might be part of the adenyl cyclase molecule. The active site of the cyclase would face the interior of the cell where it could interact with its substrate, ATP (and, perhaps, Mg^{++} and/or F^-) and the receptor or hormone-binding site would reside on the exterior surface of the membrane. Interaction of the receptor with its specific ligand (hormone) would result in a conformational alteration of the active site such that the formation of cyclic AMP would be either increased or decreased. Evidence that the sites involved in hormonal interaction and catalysis of cyclic AMP formation are distinct has been reported for the adenyl cyclases of the pineal gland[3] and fat cells.[4] It may be important to consider, however, that unlike the peptide hormones, catecholamines are small and permeable[5] and could interact with a more interior membrane site. This possibility is supported by the findings that the ability of fat cells to respond to catecholamines was significantly more resistant to digestion by trypsin than was their ability to respond to glucagon or insulin.[6] As yet there is no information about any specific biological mechanisms for abrogating the effect of adrenergic compounds on adenyl cyclase activity.

Due to the lack of precise information about the molecular events comprising an "adrenergic response", adrenergic receptors have been described and classified in terms of overall effector responses to drug administration, e. g. muscle contraction or relaxation.[7] The properties of adrenergic receptors also vary from tissue to tissue further complicating the interpretation of bioassays of adrenergic activity.[8] Stimulation of cell-free preparations of adenyl cyclase by catecholamines may be useful as the basis for a simpler and more direct assay of adrenergic activity. The most appropriate adenyl cyclase for such studies would be one derived from an accessible, homogeneous tissue; it should possess high specific

adenyl cyclase activity, stability upon storage and sensitivity to only one kind of hormonal activation. Erythrocyte adenyl cyclase possesses many of these features although the function of cyclic AMP in erythrocyte metabolism remains unknown. Erythrocytes from rats were found to contain adenyl cyclase activity which was stimulated by either F^-, catecholamines or prostaglandin E_2 but not by other hormones.[9] Isoproterenol was a more potent activator than epinephrine which, in turn, was more effective than norepinephrine. The response to epinephrine was blocked by the addition of the β-adrenergic blockers, dichloroisoproterenol and propranolol, but stimulation was also inhibited by the α-adrenergic blocker phentolamine, and by serotonin and high concentrations of methylxanthines.[10] Drug concentrations required for half-maximal stimulation of the adenyl cyclase present in rat erythrocyte ghosts were 5.0, 0.69 and 0.24 μM for norepinephrine, epinephrine and isoproterenol, respectively. Dopamine exhibited weak activity (concentration required for half-maximal stimulation: 84 μM) and no evidence was found to substantiate the existence of specific dopamine receptors.[11] Nucleated avian erythrocytes were found to contain much greater adenyl cyclase activity than their non-nucleated mammalian counterparts.[12-15] The adenyl cyclase of intact as well as cell-free preparations of avian erythrocytes responded to stimulation by catecholamines (L-isoproterenol > L-epinephrine > L-norepinephrine). Stimulation was blocked by dichloroisoproterenol but not by dibenzyline; amphetamine was inactive.[14]

A similar, highly active, catecholamine-sensitive adenyl cyclase has also been prepared from nucleated frog erythrocytes and the remainder of this chapter will be devoted to studies which pertain to its value as an assay for compounds with adrenergic activity. Erythrocytes from Rana pipiens or Rana catesbiana (commercially available from Pel-Freez Biologicals) contained adenyl cyclase activity that could be assayed in intact cells,[16] hemolysates[17] or in partially purified membrane fragments.[18] Adenyl cyclase activity was assayed with radioactive ATP (α-labelled $AT^{32}P$ or ^{14}C-ATP) in the presence of either NaF (0.01 M) or catecholamine (or analogue), Mg^{++} and Tris buffer, pH 8.1. Following termination of the reaction, $ZnSO_4$ and $Ba(OH)_2$ were added to precipitate most of the residual radioactive ATP and any ADP and AMP formed during the incubation.[19] Final purification of radioactive cyclic AMP was achieved by paper chromatography using a solvent system consisting of 1 M ammonium acetate: ethanol, 30:70 (v/v). Hemolysates or membrane fragments retained F^--and catecholamines-responsive adenyl cyclase activities during storage in liquid N_2. Very little activity could be demonstrated in the absence of either catecholamines (or active analogues) or F^-. The formation of cyclic AMP in hemolysates was stimulated by the addition of sulfhydryl compounds such as dithiothreitol and by methylxanthines. All of the adenyl cyclase activity sedimented with the membrane fraction at

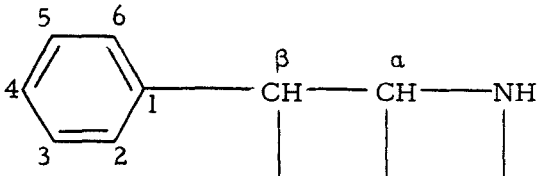
20,000 X g. Membranes which had been partially purified by passage through a CM-Sephadex column followed by repeated washings with 0.05 M Tris buffer pH 8.1, did not contain cyclic nucleotide phosphodiesterase activity and could, therefore, be assayed in the absence of methylxanthines. The addition of sulfhydryl compounds such as dithiothreitol was essential for activity.[18] Stimulation of adenyl cyclase activity by the addition of catecholamines or analogues was rapid ($\leq$ 2 min.) and proceeded in a linear fashion for 10-15 minutes. Insulin, glucagon, adrenocorticotropin, serotonin, thyroxine, triiodothyronine, prostaglandins (E_1, E_2, A_1, B_1, $F_{1\alpha}$) and vasopressin were without effect. A nondialyzable bacterial activator derived from culture filtrates of Clostridia welchii has, however, been described.[20] Some of the compounds which were able to activate or block the activation of the adenyl cyclase present in purified membrane preparations are tabulated below.[21] All of the activators had OH or CH_2OH groups in both m- and p- positions of the benzene ring, an OH substituent on the β-carbon and a primary or secondary amine. A positive correlation between potency and size of the substituent group on the amino nitrogen was noted for the compounds tested. Protokylol and isoproterenol were the most effective followed by ethylnorepinephrine and epinephrine and, lastly, by norepinephrine. The concentrations required for half-maximal stimulation were approximately 1 μM, 10 μM, and 50μM, respectively. The l-isomer of isoproterenol was approximately twice as active as an equimolar concentration of d,l-isoproterenol. The addition of α-adrenergic blockers such as phentolamine and phenoxybenzamine did not prevent activation by these compounds even though agents such as epinephrine exhibit α-adrenergic activity in other tissues. The presence of a β-OH appeared to be required for agonist or antagonist activity although the possibility that dopamine and its analogues might have exhibited activity had they been tested at higher drug concentrations ($>$100 μM) was not excluded. The following compounds had no effect upon frog erythrocyte adenyl cyclase activity when tested at concentrations between 1.0 and 100 μM: phenylethylamine, tyramine, hydroxyamphetamine, methoxyphenamine, amphetamine, methoxytyramine, dopamine, p-methoxyphenylethylamine, S35179-2, cyclopentamine, naphazoline, tetrahydrozoline, xylometazoline, mephentermine. The inhibitors or blockers of adrenergic stimulation had either m- or p- hydroxyl substituents on the phenyl group or m- and/or p- substituents other than hydroxyl groups. The phenyl group could also remain unsubstituted as in ephedrine. The potency of the blockers like that of the activators correlated positively with the size of the substituent group on the amino nitrogen. When tested against isoproterenol, propranolol was the most potent blocker followed by buphenine (nylidrin) and then, dichloroisoproterenol and sotalol. The unique potency of propranolol may be partially attributed to the separation of the aromatic moiety from the rest of the molecule by the insertion of carbon and oxygen atoms. Although most of the generalizations derived from these studies

of structure activity relationships agree with those obtained for β-adrenergic receptors in a variety of intact tissues, a few dissimilarities were also apparent. Nylidrin and isoxsuprine, for example, are adrenergic agonists in vivo although they functioned as blockers in the in vitro adenyl cyclase assay. The possibility that drugs may be modified in vivo and not in vitro must be considered. Trimethoquinone,[22] a β-adrenergic agonist with a structure that is basically different from the other compounds used, has not yet been adequately tested with this assay. The effects of all the activators were reversible and the compounds that blocked activation behaved as competitive inhibitors.

The availability of a cell-free preparation of adenyl cyclase that can be easily prepared, assayed and stored and that reacts with adrenergic compounds in a manner similar to β-adrenergic receptors in physiologically intact systems, may provide a useful tool for the design and assay of drugs with adrenergic activity and for investigating the chemical interactions between adrenergic amines and receptor molecules.

Structures of some compounds which activated or blocked adenyl cyclase activity:

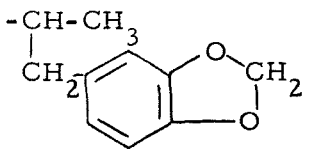
Structure



I. ACTIVATORS

Norepinephrine	3-OH,	4-OH	OH	H	H
Cobefrin	3-OH,	4-OH	OH	CH_3	H
Epinephrine	3-OH,	4-OH	OH	H	CH_3
Ethylnorepinephrine	3-OH,	4-OH	OH	H	CH_2CH_3
Isoproterenol	3-OH,	4-OH	OH	H	$CH(CH_3)_2$
Protokylol	3-OH,	4-OH	OH	H	1
AH 3365	3-CH_2OH,	4-OH	OH	H	$C(CH_3)_3$
AH 3923	3-CH_2OH,	4-OH	OH	H	2

1



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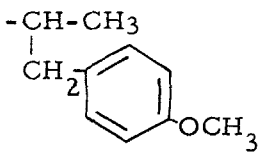
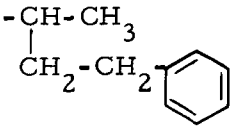


II. BLOCKERS

Dichloroisoproterenol	3-Cl	4-Cl	OH	H	$CH(CH_3)_2$
Buphenine (nylidrin)		4-OH	OH	CH_3	1.
Isoxsuprine		4-OH	OH	CH_3	2.
S 40032-7	3-OH		OH	H	CH_2CH_3
S 40045-9	3-OH		OH	H	$CH(CH_3)_2$
Propranolol		3.			
Sotalol	$4\text{-}NHSO_2$	CH_3	OH	H	$CH(CH_3)_2$
---	---	---	---	---	---
Phenylephrine	3-OH		OH	H	CH_3
Ephedrine			OH	CH_3	CH_3
S 38537-9		4-OH	OH	H	CH_3
---	---	---	---	---	---
Octopamine		4-OH	OH	H	H
Oxedrine		4-OH	OH	CH_3	H
Metaraminol	3-OH		OH	CH_3	H

1.



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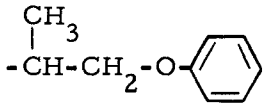

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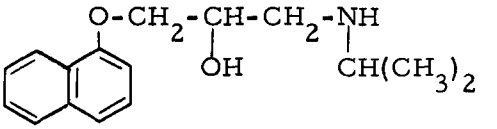

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Chapter 23. Glucagon-sensitive Adenyl Cyclase: A Model for Receptors in Plasma Membranes

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Introduction - Hormones are chemical substances capable of influencing the functional activity of certain cells but produced by cells other than those upon which they act. From this definition the concept arises that a target cell of a given hormone must contain a mechanism for recognizing and interacting with the hormone molecule and for transforming that interaction into an effect on cell function. This mechanism, generally designated the hormone receptor, has been the object of physiological and biochemical investigation throughout this century.

The discovery of the biological importance of cyclic 3', 5'-adenosine monophosphate (cyclic AMP) by E. W. Sutherland and his coworkers marked a major advance in the study of hormone action. Cyclic AMP has manifold effects upon cell function throughout the animal kingdom[1] and is produced by an enzyme, adenyl cyclase, which satisfies at least part of the definition of a hormone receptor - that of transforming the hormone-receptor interaction into an effect. The other half of the receptor definition - that of specific recognition and interaction with hormone molecules - is inferred from the fact that the activity of an adenyl cyclase system is stimulated by only those hormones which are known to affect the function(s) of the tissue of origin of that adenyl cyclase[1] but generally has not been observed directly. During the past two years, the nature of the initial interaction between certain hormones and the adenyl cyclase systems of their target tissues and the mechanism (s) by which this interaction results in increased activity of adenyl cyclase have been under investigation in this and other laboratories.

In addition to the importance of this work in endocrinology, the study of hormone-sensitive adenyl cyclase systems offers certain advantages as a model for all types of receptors, viz. hormone receptors other than adenyl cyclase, drug receptors, and neurotransmitter receptors. With an adenyl cyclase system, the consequence of events occurring at the receptor may be observed directly by following the production of cyclic AMP rather than by following some functional parameter such as muscle contraction which is at least several steps removed from the receptor and is, therefore, subject to other control mechanisms. Furthermore, recent advances in hormone chemistry and the study of biological membranes indicate that several technical obstacles in the study of adenyl cyclase systems will be overcome within the next few years.

The purpose of this review is to present the current status of one such system, the glucagon-sensitive adenyl cyclase from liver plasma membranes. Information obtained from other adenyl cyclase systems will be presented only to expand the general significance of the liver system.

General Concepts - Hormone-sensitive adenyl cyclase systems are located principally, if not exclusively, in the surface membrane of the cell[1,2,3]. Isolated fat cells contain in their surface membrane an adenyl cyclase which is activated by catecholamines, five different peptide hormones, and fluoride ion[4,5,6]. After incubation with trypsin, these cells no longer respond to the peptide hormones but do respond to epinephrine and to fluoride ion[6]. However, after incubation of "ghosts", the hypotonic lysate of isolated fat cells, with trypsin, all adenyl cyclase activity is lost[4]. These results have been interpreted to indicate a difference in accessibility of the proteolytic enzyme to the outer and inner surfaces of the plasma membrane of the intact cell and ghosts. All six hormones stimulate the same adenyl cyclase activity, but several lines of evidence indicate that they do so through distinct, specific receptors[5,6].

Based on these findings with fat cells, we have adopted the following working model of adenyl cyclase system organization. A binding site on or near the outer surface of the membrane interacts specifically with the hormone molecule. The reaction between the hormone molecule and this site leads to an increase in the activity of a separate site, on or near the inner surface of the plasma membrane, which catalyzes the conversion of ATP to cyclic AMP. Fluoride ion stimulates the same adenyl cyclase but by a mechanism which is different from that through which hormones act. A hormone sensitive adenyl cyclase system, therefore, consists of at least two functionally distinct components. It seems likely that such a system is multimolecular, but this point has not yet been proven. However, since attempts to study adenyl cyclase systems in simple, non-membraneous states have generally been fruitless, it seems prudent to consider them as complex, multimolecular structures which are intimately related to the general properties of membrane structure and function. After some comments regarding methodology, we shall proceed to examine several functionally distinct components of the liver adenyl cyclase system.

Methodology - Pure preparations of plasma membranes, free of contamination by other organelles, are ideal materials for study of hormone sensitive adenyl cyclase systems. Methods presently exist for preparation of plasma membranes from liver[7], fat cells[3], non-nucleated erythrocytes[8] and kidney[9]. Rat liver parenchymal cell plasma membranes prepared by the procedure of Neville[7] contain an adenyl cyclase which is stimulated specifically by glucagon[2]. We have recently shown that the glucagon sensitive adenyl cyclase activity of these membranes is increased 17 to 25 fold compared to a crude homogenate of liver and that the contamination by other organelles is very small[10]. Stimulation of the enzyme occurs in the presence of 10^{-10}M glucagon, is half maximal at 4×10^{-9}M, and is maximal at 10^{-7}M[10]. This dose-response relationship is identical to that of the effects of glucagon on cyclic AMP levels in a perfused rat liver system[11]. Epinephrine at 10^{-5}M produces less than 10% of the adenyl cyclase stimulation produced by glucagon at 10^{-6}M[10], but the cyclic AMP levels obtained with epinephrine in the aforementioned rat liver perfusion system are less than 10% of those obtained with glucagon[11]. The glucagon- and epinephrine-sensitive adenyl cyclase activities probably represent completely different systems in rat liver[12,13]. Plasma membranes can be prepared in large

quantities by a minor modification of the Neville method, and their adenyl cyclase activity appears to be stable indefinitely when they are stored in liquid nitrogen[10]. Other procedures are available for preparation of plasma membranes from liver, but these generally are variations on the procedures devised by Neville and offer no particular advantages for our purposes. Pure plasma membranes from other tissues either are available only in very small quantities or contain little or no hormone sensitive adenyl cyclase. For these reasons, we have chosen to use rat liver plasma membranes prepared by the procedure of Neville[7] for our studies of hormone sensitive adenyl cyclase.

Measurement of adenyl cyclase activity was a laborious and imprecise procedure until the introduction of the method of Krishna et al[14]. This method is based on the production of ^{32}P-labeled cyclic AMP from ATP-α-^{32}P. Labeled product is separated from substrate by a combination of ion exchange chromatography and adsorption of substrate to nascent $BaSO_4$ precipitate. With appropriate equipment, it is possible to perform 300 adenyl cyclase assays in one day. A complication of the method arises from the fact that adenyl cyclase preparations invariably contain sufficient ATPase activity to deplete rapidly the substrate concentration, thereby shortening the period of time over which adenyl cyclase activity can be observed and precluding even the simplest kinetic analysis. This problem has generally been overcome by addition of an ATP-regenerating system to the adenyl cyclase assay medium[10]. In addition, it has recently been shown that 5'-adenylyl-imidodiphosphate (AMP-PNP) is a substrate for adenyl cyclase but not for ATPase[15].

One method for direct investigation of the hormone-binding site interaction is to study the binding of a labeled hormone to biological materials known to contain adenyl cyclase activity which is sensitive to that hormone. Using this approach, Lefkowitz et al[16] have demonstrated binding of ^{125}I-adrenocorticotropin to an extract of an adrenal tumor, and we have demonstrated binding of ^{125}I-glucagon to liver plasma membranes. In both cases the hormones were labeled with radioiodine by the method of Hunter and Greenwood[17]. Bound and free hormone were separated in the former study either by gel filtration or by adsorption of free hormone and in the latter study by sedimentation of the membranes.

With the development of methods for plasma membrane isolation, adenyl cyclase assay, and measurement of binding of hormone to its receptor, it is now possible to study the major components of a hormone receptor.

The Catalytic Site - The adenyl cyclase of rat liver plasma membranes is a membrane bound enzyme which catalyzes the conversion of ATP (or AMP-PNP; see above) to cyclic AMP. A divalent cation, Mg^{++} or Mn^{++}, is required at the catalytic site, probably in the form of an ion-substrate complex, but the system is inhibited by Ca^{++}. Under standard assay conditions, with or without glucagon, the time course of enzyme activity is linear for at least 10 minutes and extrapolates to the origin. Activity is proportional to membrane concentration up to 1 mg of membrane protein per ml[11].

Fluoride ion stimulates the same enzyme over a range of 1-15 mM F^-; however, opposing effects of Mn^{++} and pyrophosphate on the glucagon and fluoride stimulated activities make it clear that these agents stimulate the enzyme by different mechanisms[18]. In fact, using the fluoride-stimulated activity as a marker it is possible to alter the membranes with either digitonin or phospholipase A in such a way that they retain full catalytic activity but no longer respond to glucagon[18].

Attempts to free the catalytic site of adenyl cyclase from membranes have generally been unsuccessful. However, Levey has recently described a method for solublization of cat heart adenyl cyclase by homogenization in Lubrol-PX[19]. This enzyme activity is stimulated by fluoride but not by hormones. The method has not yet been applied successfully to other tissues.

The Hormone Binding Site - Lefkowitz et al first demonstrated specific binding of labeled adrenocorticotropin to an extract of an adrenal tumor and obtained suggestive evidence that this binding was related to the activation of adenyl cyclase[16]. We have subsequently demonstrated binding of labeled glucagon to liver plasma membranes[20]. This binding is diminished by dilution of the labeled material with unlabeled glucagon but not by dilution with insulin, secretin, adrenocorticotropin, or other peptides and is, therefore, specific for glucagon. The patterns of glucagon concentration dependence appear to be identical for glucagon binding and for activation of adenyl cyclase. Modification of glucagon by exposure to liver plasma membranes alters the binding and adenyl cyclase activating properties of glucagon identically. Finally, modification of the membranes with urea, digitonin, or phospholipase A alters both the ability of the membranes to bind glucagon and to respond to glucagon stimulation of adenyl cyclase activity[20]. These several lines of evidence strongly suggest that the observed binding of glucagon is related in some way to the process of activation of adenyl cyclase by glucagon.

Binding of glucagon to liver membranes occurs very rapidly, but 5 to 15 minutes are required for maximal binding to occur. Methodologic limitations have precluded a meaningful comparison of the kinetics of glucagon binding and adenyl cyclase activation. Under ordinary conditions, 10 to 30% but never more than 40% of the labeled hormone added is bound. The failure to achieve complete binding of the hormone is probably due to simultaneous inactivation of the hormone (see below) which occurs during the binding incubation. The binding is temperature dependent with the binding measured at 0° being about one-third that observed at 30°. Binding occurs in a simple buffered albumin medium. No co-factor requirements have yet been identified[20].

Under minimal conditions, membranes and labeled glucagon incubated in buffered albumin, the binding of glucagon is not fully reversible. If a 1000 fold excess of unlabeled glucagon is added to a binding incubation after a period of time sufficient to permit maximal binding of labeled glucagon and incubation is then continued for up to several hours, only a small fraction of the labeled glucagon is displaced from the membranes by

the unlabeled glucagon[20].

The Interaction Between Glucagon and its Binding Site - The interaction between a hormone and its receptor must ultimately be described in terms of specific intermolecular forces between the two structures. This ideal has not been achieved in the case of polypeptide hormones for many reasons, not the least of which is the complexity of peptide hormone molecules. Fortunately, several fragments of glucagon have recently become available, and these have provided important clues regarding intermolecular forces.

Glucagon is a single peptide chain of 29 amino acids. Most of the usual α-amino acids are represented, but cystine, cysteine, and proline are notably absent. No unusual amino acids are present. The amino terminus is histidine, and no other histidine residues are present. A region near the center of the molecule contains a high concentration of amino acids which would probably be charged at neutral pH. The region from residue 20 through residue 27 contains a high concentration of non-polar residues (for details and rational of this analysis, see reference 21).

If the amino terminal residue, histidine, is removed from the glucagon molecule, the resulting fragment, des-1-histidine-glucagon (DH-glucagon.) does not stimulate adenyl cyclase activity but is a competitive inhibitor of glucagon stimulated adenyl cyclase activity and of glucagon binding[21]. Inhibition studies indicate that the apparent affinity of the system for DH-glucagon is 10-20 fold lower than for glucagon. Thus, the amino terminal histidine is essential for biological activity and contributes to, but is not essential for, binding of the glucagon molecule to its receptor. Histidine residues have been found at the active centers in several peptides and in hemoglobin, and its role at the active site in the glucagon-receptor complex may be similar to its role in one of these proteins.

If two amino acid residues are removed from the carboxy terminus of the glucagon molecule, the resulting fragment retains biological activity [22]. However, if glucagon is cleaved between the 21st and 22nd amino acid residues, both fragments are biologically inactive and neither will antagonize the stimulation of adenyl cyclase by glucagon or the binding of glucagon to liver membranes[20,21]. Thus, most of the glucagon molecule is required for expression of its biological properties, and the hydrophobic region near the carboxy terminus is necessary but not sufficient for binding of the molecule to its receptor.

The lipoprotein nature of the glucagon binding site (see below) and the importance of the hydrophobic region near the carboxy terminus of glucagon suggest that part of the interaction between glucagon and its receptor may be hydrophobic in character. The reduction in binding observed upon incubation at 0° or in the presence of relatively low concentrations of urea may also indicate hydrophobic interaction[21]. A separate and convincing line of evidence on this point is the observation that detergents and phospholipids bind strongly to the carboxy terminal region of glucagon and alter the tertiary structure of the pep-

tide[23]. Finally, the residue 22 through 29 fragment of glucagon is extremely insoluble in water but dissolves readily in methanol. The total energy available in the form of hydrophobic bonds involving the carboxy terminal region may provide a substantial part of the forces which binds glucagon to its receptor, or, stated another way, if both glucagon and its binding site when separated have large hydrophobic areas exposed to an aqueous medium, the complex of the two structures would be thermodynamically more stable.

Another method of analysis which may provide useful clues to the intermolecular forces involved in the glucagon-receptor complex is a comparison of the primary structures of glucagon and a very similar peptide hormone, secretin. The sequences of the two hormones are:

Glucagon[21]: H-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-
// // // // // // :: ? // :: ? //
Secretin[24]: H-His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Asp-

Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-OH
// // :: // :: :: // ? // ::
Ser-Ala-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH_2

In this comparison, identical residues in the two sequences are indicated by // between the residues, those with similar polarity by ::, those with uncertain similarity by ?, and by no symbol where the residues are dissimilar. It is apparent that there are only seven dissimilar or uncertain pairs in the two sequences. Yet glucagon and secretin have different receptors in fat cells[6], and secretin neither stimulates adenyl cyclase nor antagonizes the glucagon stimulation of adenyl cyclase[10] or glucagon binding[20] in liver membranes. An investigation of what changes in sequence are required to make secretin behave as glucagon and vice versa should reveal which residues confer this specificity and provide information regarding the intermolecular forces between these hormones and their receptors.

The importance of secondary and tertiary structure in the glucagon molecule has not yet been established. Glucagon is probably randomly coiled in dilute solution[25,26], but is 75% α-helical in concentrated solution or in the crystalline state. Helix formation in the molecule is thought to be related to the arrangement of hydrophobic residues[27]. The tertiary structure changes induced by binding of detergents to glucagon (see above) suggest that the tertiary structure of glucagon in aqueous solution may be different from that of glucagon bound to its receptor.

A Possible Regulatory Site for Nucleotides - In the course of preliminary characterization of glucagon binding, significant differences in binding were observed in media containing or not containing the substrate and cofactors for the adenyl cyclase reaction, ATP, $MgCl_2$, ATP-regenerating system, etc.[28]. Three specific differences were noted: (1) the maximum amount of glucagon bound was less in the presence of adenyl cyclase reagents than in their absence, (2) the time of incubation required to

achieve maximal binding was less in the presence of adenyl cyclase reagents, and (3) labeled glucagon bound to membranes could, in the presence of adenyl cyclase reagents, be displaced by addition of a large excess of unlabeled glucagon. Elimination experiments led to the conclusion that ATP was responsible for all three effects[28]. These effects of ATP were subsequently found to be non-specific in that several other nucleotides, at sufficient concentration, could mimic them; however, GTP and GDP were found to be much more potent than all others tested. Effects of GTP and GDP could be detected at concentrations as low as 10^{-8}M and were half-maximal at about 10^{-6}M; GMP, AMP, cyclic GMP, and cyclic AMP were found to be ineffective. Thus it appeared that certain nucleotides, particularly ATP and GTP, could influence the binding of glucagon to its receptor.

In order to test the effects of nucleotides on the stimulation of adenyl cyclase activity by glucagon, a major technical obstacle had to be overcome. ATP, at the concentrations usually employed in adenyl cyclase assay media, is capable of producing near maximal nucleotide effect on glucagon binding. Since adenyl cyclase cannot be assayed in absence of its substrate, extraordinary conditions had to be employed for evaluating the effects of nucleotides. Fortunately, adenyl cyclase activity can be observed at ATP concentrations lower than the minimal concentrations required to affect binding. When ATP concentration in the adenyl cyclase assay medium was reduced to about 0.1 mM, GTP was found to increase the glucagon stimulated adenyl cyclase activity. Unfortunately, the ATP-regenerating system was found to be ineffective at these low ATP concentrations, and GTP tended to preserve substrate concentration under this condition. Shortly after these experiments were performed, AMP-PNP was found to be a substrate for adenyl cyclase activity[15]. Since AMP-PNP concentration is not diminished upon incubation with plasma membranes, effects of nucleotides could be investigated without concern for artifacts introduced by preservation of adenyl cyclase substrate concentration. Conditions were shortly established wherein no increase in adenyl cyclase activity due to addition of glucagon could be observed unless a nucleotide was added to the assay medium[25]. Thus it is clear that a nucleotide, possibly GTP, is obligatory for glucagon stimulation of adenyl cyclase activity.

A Possible Regulatory Site for Magnesium - A divalent cation, either Mg^{++} or Mn^{++} is required for catalytic activity of adenyl cyclase[10]. Since ATP binds one Mg^{++} at neutral pH, it is reasonable to assume that the adenyl cyclase substrate is actually MgATP[4]. Kinetic analysis of adenyl cyclase activity in fat cell ghosts[9] and an extract of heart muscle[29] suggested that Mg^{++} might also be required at a second site. In homogenates of parotid gland[30] and brain[31] and in liver membranes[32], fluoride irreversibly activates adenyl cyclase activity only when Mg^{++} is also present. The complexity of the membranes and of the adenyl cyclase assay medium have forced postponement of further evaluation of the role of magnesiums and other ions.

A Role for Membrane Lipids - By careful selection of conditions, it is possible to alter liver membranes with either digitonin or phospholipase A in

such a way that glucagon stimulated adenyl cyclase activity and glucagon binding is markedly diminished but fluoride stimulated adenyl cyclase activity is either unaffected or increased[18]. Addition of aqueous dispersions of membrane lipid extracts or purified phospholipids partially restores the glucagon stimulated adenyl cyclase activity and glucagon binding[33,34]. The glucagon response of a solublized adenyl cyclase from heart muscle can be restored by addition of phosphatidyl serine[35]. These results clearly establish the importance of membrane lipids in the process through which glucagon stimulates adenyl cyclase activity; however, the mechanism of lipid involvement is not known. In view of its susceptibility to detergents and phospholipase A, it is probable that the glucagon binding site is a lipoprotein. However, evidence for lipid involvement at other sites in the system is available[34]. The glucagon binding site and the adenyl cyclase catalytic site are probably located on the outer and inner surfaces of the plasma membrane respectively. Plasma membranes consist of one-third to one-half lipid[36,37], and several lines of evidence indicate that there are extended lipid-rich regions in membranes[38]. Thus, it may be that the hormone binding and catalytic sites of the adenyl cyclase system are separated by a lipid-rich layer. A suggestion has recently been made that lipids act by limiting adenyl cyclase activity and that hormones act by relieving this limitation[31].

The Coupling Mechanism - The most interesting and poorly understood property of hormone sensitive adenyl cyclase systems is the process by which the hormone-binding site interaction leads to increased catalytic activity of adenyl cyclase. The best current hypotheses are that the interaction at the binding site produces either a conformational change analogous to those known to occur in allosterically regulated enzymes or a covalent change such as the production of a phosphorylated intermediate. Compelling evidence for or against either hypothesis is not yet available. Phosphorylation seems unlikely because glucagon stimulated adenyl cyclase activity can be observed in the absence of an added source of high energy phosphate when AMP-PNP is used as substrate[15]. However, other kinds of covalent change remain entirely possible. The coupling mechanism must operate very rapidly since the time course of glucagon stimulated adenyl cyclase activity in liver membranes is linear for at least 10 minutes and extrapolates to the origin[10].

GTP stimulates adenyl cyclase activity in the absence or presence of added glucagon[15] and alters the binding of glucagon to the membranes[28]. Furthermore, a requirement for a nucleotide must be fulfilled in order for glucagon to stimulate adenyl cyclase activity[15]. If all of these effects are caused by an interaction between nucleotides and single extracatalytic site, it is attractive to think that this site and events occurring at it form part of the coupling mechanism. A requirement for lipids in the process through which hormones stimulate adenyl cyclase activity has been established[34], and the hypothesis has been advanced that hormones act by relieving a limitation on adenyl cyclase activity caused by lipids[31]. Thus, protein-lipid interactions may also be involved in the coupling process. All of these considerations about the coupling between the glucagon binding site and the adenyl cyclase catalytic site remain specu-

lative. Although experiments with intact membranes can provide indirect information regarding this process, intimate understanding of its details will ultimately require simplification of the system and separation of its components. Thus the problem is inextricably related to a general and very difficult current problem in biology, that of resolving membrane structure and function.

Termination of the Glucagon Signal - In order to subserve a regulatory function, a hormone receptor must be able both to turn on and to turn off in response to increasing and decreasing blood hormone concentrations. If a large excess of DH-glucagon, a competitive inhibitor of glucagon (see above), is added to an adenyl cyclase reaction which has been allowed to incubate for several minutes in the presence of a submaximal stimulating concentration of glucagon, the rate of production of cyclic AMP decays to the basal level within one minute[32]. Thus, the activation of adenyl cyclase depends from moment to moment on the composition of the medium rather than on the state of system in the immediate past.

Glucagon in the medium of an adenyl cyclase reaction is very rapidly inactivated by a membrane dependent process[20]. The inactivation process is specific for glucagon since it cannot be blocked by addition of a large excess of another peptide hormone such as secretin. In common with the glucagon stimulated adenyl cyclase, the inactivation process is heat labile, stimulated by EDTA, and inhibited by 2 M urea. The change in the glucagon molecule has not been determined, but it must be very minor since the inactivated glucagon co-chromatographs with active glucagon on sephadex, DEAE cellulose, and a thin layer partition chromatography system[32]. Thus the liver plasma membrane, an organelle which contains the glucagon receptor, also contains a specific glucagon inactivation system which shares several properties with the receptor. The inactivation system may be a part of the receptor, in which case glucagon would actually be a substrate for the receptor, or it may be separate and serve the function of rapidly reducing the concentration of hormone in the vicinity of the receptor once the supply of new hormone has diminished.

General Conclusions and Summary - The glucagon-sensitive adenyl cyclase system in purified rat liver plasma membranes has properties which satisfy the major criteria required to call it a glucagon receptor. It is capable of responding very rapidly to changes in glucagon concentration and is sensitive to very low concentrations of the hormone. It produces a substance, cyclic AMP, which affects cellular function in ways which would be predicted from the known physiologic effects of glucagon, and the system displays great specificity for glucagon. Furthermore, in the liver plasma membrane preparation, the system remains associated with the organelle in which it probably resides in the living cell but has been separated from most of the extraneous cellular material. Thus the system is probably in the simplest possible state obtainable without disrupting the organelle structure.

The glucagon-sensitive adenyl cyclase system is clearly complex and probably multimolecular; but a general formulation of its operations can

be made. Glucagon binds tightly but reversibly to a specific site on the outer surface of the membrane. The binding site has the properties of a lipoprotein, and part of the force of binding involves a hydrophobic interaction between the binding site and the highly nonpolar carboxy terminal region of the glucagon molecule. A comparison with a structurally similar but inactive peptide, secretin, indicates that the specificity of the binding is due to one or more of seven amino acid residues which are located mainly in the mid-portion of the molecule. The amino-terminal histidine is essential for biologic activity but not for binding and therefore must play a unique role at the active center of the glucagon-binding site complex. The change in both structures consequent to the reaction between glucagon and the binding site is not known but tertiary structure changes occur in both, and covalent changes in either or both may occur as well. The changes occurring upon reaction at the glucagon binding site cause an instantaneous increase in the activity of the adenyl cyclase catalytic site on the inner surface of the membrane. The nature, or even the complexity, of this coupling reaction has not been established but a nucleotide, probably either ATP or GTP, must be present in sufficient concentration for coupling to occur. In addition, lipids are present between the hormone-binding and catalytic sites and may contribute to the coupling mechanism. The substrate of the catalytic site is Mg-ATP, and the products are cyclic AMP and PP_i. Mg^{++} is also required at an extracatalytic site which may subserve a regulatory function. Intracellular events are probably able to regulate the response of the system to hormonal stimulation through either or both the magnesium and nucleotide sites. The entire system responds very rapidly to changes in the medium and will decay to a lower level of activity within one minute after reduction of the medium glucagon concentration. The membrane contains a specific system which produces a minor change in the glucagon molecule and renders it inactive, thereby rapidly reducing the effective glucagon concentration after cessation of supply of new hormone to the system.

This summary of the glucagon sensitive adenyl cyclase is still somewhat speculative and contains several important gaps. However, it is the only available synthesis of the operations of a complete hormone receptor and, as such, offers a framework for experimental testing of its parts. Much of this work can be done with membranes. For example, the generality of the model should be examined, and it should be possible to identify the product of the glucagon inactivation reaction. However, such experiments are limited in scope, and full understanding of the systems will ultimately require separation of its components from the membrane structure. In particular, the nature of the coupling process probably will not be clarified until the system is simplified. When such information becomes available, it must be incorporated into an understanding of the system in its natural membraneous state just as events occurring at the membrane level must eventually be incorporated into an understanding of the functions of the whole organism.

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Section VI - Topics in Chemistry

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Chapter 24. Quantitated Structure-Activity Relationships

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An increasing number of persons are beginning to apply quantitated structure-activity relationships (SARs) as an aid in the study of factors influencing drug effect and as a guide for the design of new drug agents. Pioneering studies discussed in previous ANNUAL REPORTS[1-5] and in several recent reviews[6-8] have indicated the potential utility of these approaches and efforts are currently being made to refine the present methods and to extend their practical utility. Three basic methods are in current use: (1) Linear Free-Energy and Related Mathematical Models; (2) Polarizability Models; and, (3) Quantum Chemical Models. These methods differ in the level of theoretical sophistication needed to obtain a working relationship, but all presently rely heavily on the use of multiple regression techniques in relating observed biological activities to a given mathematical model. For the novice, a slight change in perspective is necessary to gain an appreciation of the intent and utility of quantitated methods in application to biology. The intent is not simply to correlate biological data with physical constants, nor is it the a priori determination of the biological activity that should be observed for a given compound. Rather, the objectives of quantitated SAR studies are to take concepts on how molecular properties are said to influence a given biological effect and to relate these concepts to a mathematical, physical or chemical model. In this way an expression based on a physicochemical theory can replace a verbal rationale for experimental observations. If the working theory is well-founded, derivation can supplement or at times replace intuition in providing an indication of the course to follow in conducting later experiments with the same test system.

In seeking new drug agents three procedures are often followed: (a) the routine screen, where compounds from varying sources are tested for their effect against a predetermined disease or disease state; (b) structural modification, where compounds whose biological effects are known are used as models for the design of new compounds having a similar biological effect; and, (c) biochemical design, where a compound is patterned to act in lieu of or in a manner similar to a known biochemical substance. Lead substances garnered from the results of these studies provide models for subsequent structural variations which are often prepared with a view towards gaining the biologically most potent representatives of the series. It is here where quantitated SARs are currently most useful, although the methods are potentially of broader scope.

Linear Free-Energy and Related Mathematical Models. The most direct connection between an intuitive interpretation of a given set of SARs and its

mathematical counterpart is the use of the additive statistical model.[9,10] By inspection of a table of SARs it can often be concluded that the incorporation of certain groups which seem to enhance a biological effect into a single compound might lead to a more active agent. If the set of SARs is extensive, however, the most suitable combination of substitutions is often not obvious and may not even be possessed by any of the compounds contained in the set. The additive model represents an effort to replace the method of inspection by a mathematical procedure which is operationally equivalent but which has the advantage of rapidly pointing out those groups which seem most likely to lead to an optimal biological effect.

The additive statistical model can be represented by the equation

$$A = a_{pm} + a_{qn} + \ldots + \mu$$

where A is the observed biological activity for a compound and a_{pm}, a_{qn} are the relative magnitudes of the biological effect imparted by substituents m and n at molecular positions p and q, respectively, measured with reference to a standard activity μ. There is an equation of this type for each compound in a set of SARs. The reference activity μ may be taken as the average of the biological activities for all compounds in the SAR compilation or it may be chosen as the observed biological activity for the parent member of the series. Values for a_{pm}, a_{qn} are subsequently derived by least-squares procedures and these provide a measure of the influence of each structural modification on the observed biological effect. Those substitutions leading to enhanced biological activities, as indicated by positive a values, are the more promising structural variations for later synthesis. The magnitude of the biological effect to be expected due to the incorporation of these "optimal" structural variations is obtained by adding the appropriate derived values of a to the value for μ. Details of the computational technique will be found in a forthcoming book edited by Kier.[11]

Purcell and his coworkers have been active in attempting to define the conditions under which the application of an additive model to biological data might break down.[12,13] At present, the demonstrated limitations are primarily statistical in origin. A procedure for obtaining reliable a values illustrated for chloroquine derivatives tested against *Plasmodium gallinaceum* is given by Hudson *et. al.*[14]

While use of the additive model possesses a definite advantage over visual analyses of SARs, it is restricted in the same ways as is the inspection method. It is of limited utility in providing insight into the physical or chemical drug requirements which limit a biological response and it is applicable only to those substituent variations contained in the SAR compilation. Recent work indicates, however, that a values identified with a given molecular position may be correlated with a linear combination of electronic, lipophilic and steric substituent parameters. Two illustrations are provided by the work of Cammarata and Yau[15] in which a values derived from microbial growth kinetic assays of tetracyclines are related to σ^2 and r_v and by the work of Fujita and Ban[16] in which a values derived from enzymatic assays of phenethylamines are correlated with σ and π. The

substituent constants involved are σ, the Hammett constants, π, the Fujita-Hansch-Iwasa lipophilic indexes,[17] and r_v, the minimum van der Waals radius for substituents.[18-20] Further developments along these lines could extend the utility of additive statistical analyses since new a values can be determined from a knowledge of the values for appropriate substituent constants and the correlation equation.

In the linear free-energy approach developed by Hansch and his associates[2,3] biological activity is treated as a free-energy related property. The model equation used in this approach may be generalized by the relation

$$A = a\sigma + f\sigma^2 + b\pi + c\pi^2 + d\, r_v + g\, r_v^2 + k.$$

Higher power (squared) terms appear in this equation to take into account the possibility that the biological activities for a series of compounds may pass through a maximum in relation to a given substituent property. As this behavior is usually a consequence of lipophilicity a reduced form of the linear free-energy model is often sufficient

$$A = a\sigma + b\pi + c\pi^2 + d\, r_v + k.$$

The inclusion of higher power terms in the linear free-energy model is a statistical expedient for arriving at correlations of biological data. The Higuchis[21,22] have developed a thermodynamic model which accounts, in part, for the need for higher order terms, especially with reference to lipophilicity, but their method is more difficult to apply for an initial analysis of biological data.

The application of the linear free-energy approach is usually restricted to compounds which are structurally closely similar, but in cases where a biological effect is largely limited by the lipophilic characteristics of the drug agents this restriction may not pertain. Interpretations of a correlation gained by this approach are based on the principle that a parallelism should exist between physical parameters derived from a well-characterized model process and the corresponding physical influence that may dominate in the actual process under study. Since a rationale can usually be presented which is consistent with a correlation involving physically meaningful parameters, it has been pointed out that care should be taken to insure that an initial correlation is not a statistical artifact. Martin[23] has indicated the consequences due to rounding errors and Cammarata et. al.[24] have presented examples where correlations of biological data are insufficient to justify a physical interpretation.

Craig and his associates,[25] in an interesting case history, discuss the significance of the linear free-energy approach in relation to a program dealing with the development of 3-tropanyl 2,3-diarylacrylates as spasmolytics. Based on the finding that the spasmolytic potencies for a representative series of 20 compounds was correlated with σ and σ^2 further work on this series was terminated. These workers felt there was a high probability that an outstandingly active molecule in this series will not have been

missed by not preparing "just a few more analogs" since the optimum activity for the series is already indicated by the correlation.

A study made by Kutter et. al.[26] indicates a potential for separating drug penetration effects from effects due to an actual drug-receptor combination. Analgetic efficiencies of morphine-like drugs were determined following intravenous and intraventricular dosing of rabbits. It was shown that the ratio of threshold doses, $\log(C_{iventr}/C_{iv})$, is related to the ability of the drugs to penetrate the lipophilic blood-brain barrier. In contrast, the intraventricular activities were suggested to be more in parallel with the "receptor activities" of the analgetics since these activities were essentially independent of the lipophilicity of the compounds.

Hansch and Coats[27] have analyzed data from a number of laboratories in mapping the physical characteristics for the active site of α-chymotrypsin. In accord with the Hein-Niemann model 4 physically distinct regions on the enzyme were identified as common to both substrates and inhibitors. Extrapolations of this study may be to take any of a number of proteins and enzymes whose primary and tertiary structures are known and to design specific substrates or binding agents based on a knowledge of the macromolecular structure. From such pursuits rules applying to quantitated SARs in relation to the nature of the active or binding sites of macromolecules might be derived which might prove useful in understanding the nature of other, less well-defined, receptor regions.

Drug effects potentially due to the intermediacy of free-radicals are presently under investigation using linear free-energy approaches.[28] One illustration of the uncertainties involved in this area of study is provided by work done in relation to the bacterial growth inhibition potencies of chloramphenicols. Cammarata[29] has reported a correlation of these activities in which electronic polarizability was suggested as a biologically limiting physical property. Hansch et. al.[30] subsequently found the same data can be correlated by an equation involving E_R, which is defined as a free-radical parameter, and attributed the antibacterial effect of chloramphenicols to the formation of a benzylic radical. Later Cammarata and coworkers[31] showed E_R is correlated with σ^2 and concluded that the physical significance of E_R is obscure. From cell-free studies Freeman[32,33] found no justification for interpreting the action of chloramphenicols based on a correlation of antibacterial activities. His data for the inhibition of bacterial protein synthesis by chloramphenicols suggest the penetration characteristics of these drugs are sufficient to account for variations in their antibacterial effects.[32] Whether Freeman's conclusion applies generally to the use of linear free-energy analyses as a mechanistic tool in biology or whether it serves to point out a possible pitfall of the approach in this connection should be established by further, well-designed investigations.

A number of other linear free-energy analyses have appeared in the recent literature dealing with enzyme inhibition,[34-42] antibacterial,[43-48] antiparisitic,[49,50] antitumor,[51] fibrinolytic,[52] and hemolytic[53] activities. Various other biological activities, such as odor and taste,[54,55] have also

been similarly studied.[56-58] It is not intended that the positive contributions made in these studies be minimized in this necessarily brief account. Those examples which have been presented are representative of the work done and yet to be done with biological linear free-energy relationships.

Polarization Models. In some instances the use of a linear free-energy model fails to lead to a correlation of the biological data despite the variety of substituent constants that may be tried. One rationale for this finding is that a factor not encompassed by the more usual substituent constants may be the biologically limiting physical influence. For these instances an approach based on the theory of intermolecular forces, an elementary discussion of which is given by Hildebrand and Scott,[59] could pertain. Tute has outlined this theory as applied by McFarland to biological systems.[60] The model equation used in this model, written in a form suitable for regression analysis, can be expressed

$$A = a\mu + b\mu^2 + c\alpha + k$$

where μ is the dipole moment and α is the polarizability for a molecule. Tute has used this model in correlating the viral neuraminidase activities for a series of isoquinolines.[60] Presently, however, the usefulness of the polarization model in relation to biological systems is not firmly established.[24]

Quantum Chemical Models. One of the more difficult methods to apply to pharmacological systems, and also one which is difficult to appreciate by many, is based on solutions to the Schroedinger equation for molecular systems. This type of an approach is general in the sense that all physical and chemical molecular properties have an electronic origin, but it is restricted operationally by the nature and number of assumptions that may have to be made in order to reduce the mathematics to a form that is readily applied. A further more practical restriction is the computer cost, which can be huge in comparison to that for the application of an alternative model. These restrictions can be made at least more bearable by (a) selecting the quantum chemical method giving the most adequate description of the molecular properties of interest for the least cost and (b) using a quantum chemical approach if the insight that might be gained into a biological process is either difficultly or not at all accessible experimentally. Much of the current research in quantum pharmacology, as this area may be termed, is exploratory in relation to the study and design of drugs. The results obtained to this date are promising. A recent book by Kier[61] details many of the methods and implications of this approach.

Computational studies made by Kier and his coworkers, most recently on amino acids,[62,63] dopamine,[64] thyroxine,[65] oxotremorine,[66] and phenyl choline ether,[67] are close to views shared by medicinal chemists since the calculations deal with the conformations of drug agents. Work such as Kier's is valuable since not only can the most stable conformations accessible to a drug molecule be calculated (these can frequently be determined by X-ray or n.m.r. techniques) but also less stable, potentially undetected conformations available to a molecule could be indicated. At least one of the

conformations accessible to an unbound drug could control the nature of the initial interaction between the drug and its receptor[64] and may at times be similar to the conformation of the bound drug. Gass and Meister[68] have made empirically-based calculations in an effort to describe the conformation of glutamate as bound to the active site of glutamine synthetase. Parallel studies using quantum chemical methods may provide an indication of the conformation of drugs bound to their receptor.

Peradejordi et. al.,[69] Wohl,[70] and Bass et. al.[71] have developed models based on quantum chemical considerations which are suited to the analysis of biological activities by multiple regression techniques. The work of Peradejordi and his associates[69] is noteworthy for its efforts towards theoretical rigor. The regression model developed in the latter study is given by

$$A = \sum_{i} (a_i Q_i + b_i S_i^E + c_i S_i^N) + k$$

where Q is the net charge, S^E is the electrophilic and S^N the nucleophilic "superdelocalizability" of an atom. The summation is taken over all atoms of a drug molecule and regression methods are used to determine which atoms have their properties related to the drug effect under study. The in vitro bacteriostatic activities of tetracyclines,[69] the antihypertensive activities of benzothiadiazines,[70] the antimalarial activities of chloroquine analogs,[71] and various steroidal activities[72] have recently been analyzed using this type of method. In each case the drug atoms which seem most essential in gaining a given biological response are identified. Conceivably new molecules of similar steric and lipophilic nature having a distribution of atoms like that reflected by the correlations could lead to an equivalent biological effect. Efforts have been made to incorporate lipophilic influences into this approach.[71,73,74]

Storm and Koshland[75] followed by Milstien and Cohen[76] have presented chemical evidence suggesting that orbital directional characteristics are a dominant and possible major cause of the catalytic efficiency of enzymes. No doubt orbital symmetry control of many enzymatic and pharmacological processes can be encompassed by the Woodward-Hoffman rules,[77,78] but it remains to be shown how these rules can be generally applied to biological systems. A fundamental understanding of biological specificity at the receptor level is the potential offered by developments in this area.

An electron transfer mechanism modified by steric influences is suggested by Kang and Green[79] as accounting for the hallucinogenic activities of the major drugs of abuse. Testable predictions expedited by molecular orbital calculations have been presented.[79,80] An energy transfer mechanism similar to that which has been used to gauge the active center of carboxypeptidase A[81] has been proposed as a basis for the anti-inflammatory activities of nonsteroidal agents.[82] The significance and utility of this latter course of investigation is open to conjecture.

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Chapter 25. Pharmaceutics

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Membrane Transport - A knowledge of the factors affecting the permeability of membranes to solutes is crucial to the development of theoretical models to describe a variety of processes of interest to pharmaceutical scientists. Diffusion through membranes has obvious implications in the areas of drug absorption, the release of drugs from coated tablets and capsules, and the degradation of compounds which results from the penetration through protective coatings of water vapor and other gases.

A number of studies have been undertaken to establish an experimental model for investigating the influence of various barriers on the rate of drug transport. Herzog and Swarbrick[1] have constructed a polymeric, nonporous, model membrane consisting of 44% ethylcellulose, 44% biological materials including lecithin, *cephalin* and cholesterol, and 12% mineral oil. When tested in a two-compartment transport cell using salicylic acid as penetrant, this membrane was found to mimic the functionality of natural barriers. The transport of salicylic acid follows apparent first-order kinetics, with the membrane retaining approximately 2% of the salicylic acid. Both lecithin and mineral oil are found to potentiate transport, apparently due to their contribution to the nonpolar character of the barrier.

Nakano and Patel[2] have observed that the permeability of a diffusate through a nonpolar (dimethyl polysiloxane) membrane is increased in the presence of complexing agents which interact with the diffusate in nonpolar environments, but is decreased in the presence of those agents which complex with the diffusate mainly in aqueous solution. In the case of complexing agents which interact both in aqueous and in nonpolar environments, either a decrease or an increase in permeability may be observed, depending on the partitioning behavior of the complexing agents and the relative stability constants of the complexes formed in the two environments.

In a similar study,[3] an olive oil impregnated Millipore membrane was employed to determine the transport kinetics of several drugs between two aqueous compartments buffered at pH 1.2 and 7.4. Apparent first-order rate constants obtained for the drugs alone can be correlated with reported in vivo absorption rate constants. Each of the drugs is known to complex with caffeine, and in each case caffeine significantly reduces the rate of transport. Furthermore, calculated in vitro rate constants agree well with reported in vivo rate constants for the drug-caffeine complexes.

The transport cell devised by Nogami and coworkers[4] consists of three compartments partitioned with two semipermeable (Visking) membranes, the central compartment being thin and containing a solution of polyvinylpyrrolidone, which

serves as a nonpermeating third component. Equations were developed to express the relationship between the overall permeability constant and the permeability and transport constants of the individual liquid and membrane barriers. After a short induction period, a quasi-steady state condition is reached when barbital and benzoic acid are employed as the diffusible solutes. Sodium chloride has a negligible effect on the permeation of barbital and benzoic acid. The thickness of the central compartment apparently does not influence the rate of permeation since penetration through the Visking membranes is rate-determining. The interactions between the drugs and PVP can be described by a modification of the Langmuir adsorption equation.

Ghanem and coworkers[5] have examined the influence of surfactants, electrolyte type, and concentration upon the permeability coefficient of the interfacial barrier formed when gelatin is adsorbed at the hexadecane-water interface. Using diethyl phthalate as the solute, they found that ionic surfactants increase the permeability of the barrier. Salts also increase the rate at which diethyl phthalate penetrates, but the effect can be attributed entirely to an alteration in the partition coefficient of the solute, and not to an effect on the permeability. Thus, in spite of gelatin's polyelectrolytic nature, its configuration at the interface appears to be little affected by variations in the salt concentration. This is in contrast to the finding of Dorst and coworkers[6] that the penetration of ethanol through a series array of membranes consisting of a highly crosslinked ion exchanger is influenced by sodium chloride.

The relative importance of oil-water interfacial barriers and bulk diffusion in complex matrices has been compared by following the transfer of cholesterol, diethyl phthalate, progestrone and octanol: (1) from water into hexadecane droplets in a continuous encapsulating gelatin layer or matrix, and (2) into and out of aggregated hexadecane-containing gelatin capsules dispersed in water. Experimental results obtained for cholesterol support a mechanism in which the interfacial barrier is rate-determining for both systems. In the case of the other solutes, the oil-water interfacial barriers are found to be controlling in the experiments with aggregates, but bulk matrix diffusion factors, as well as the oil-water interphase transport, are found to be important in the case of continuous matrix layers.

Sustained Release - Fundamental information concerning membrane transport is likely to find ready application in the development of more effective forms of sustained release medication. The permeability of films composed of poly(methylvinylether)-maleic anhydride copolymer crosslinked with polysorbate 20 can be altered by an adjustment of the polysorbate 20 content, by varying the molecular weight of the polymer, and by selecting the appropriate humidity pretreatment procedure.[8]

The incorporation of drugs into polymeric matrices is an alternative device for controlling release. Among factors found to influence the release process from such matrices are the partition coefficient and diffusion coefficient of the drug, and its

concentration within the polymer. Using medroxyprogrestrone acetate as the diffusible species, Roseman and Higuchi[9] found a nonlinear dependence of release rate upon concentration of the compound in the matrix. Equations have been derived to explain this behavior, and to incorporate other parameters which may influence the release rate. The model employed depends upon a receding medroxyprogrestrone acetate layer within the matrix, and the validity of the model was substantiated by use of photographs depicting depletion zones as a function of time.

The phenomenon of flocculation in polymeric systems has been evaluated for its *potential* as a means of facilitating the molecular entrapment of drugs. Highly concentrated colloidal polymeric dispersions of an acrylic copolymer are flocculated in the presence of a drug such as methapyrilene hydrochloride in solution.[10,11] A suitable organic acid greatly increases the degree of interaction between the drug and the polymer, and provides a mechanism for controlling both interaction and subsequent drug release properties. Variables such as flocculation pH and rate of agitation must be controlled. Given the water solubility of a drug and its affinity for the polymer phase it should be possible, at least in theory, to design a polymer network having optimum release properties.[12]

Dissolution and Solubility - The search for apparatus which will afford reliable and reproducible information concerning the dissolution of pharmaceutical dosage forms continues. Swarbrick[13] has reviewed the various theoretical models that have been proposed to describe the dissolution process, as well as the devices used in its measurement. Wagner[14] has also published a comprehensive review of this field. Others[15-18] have tested dissolution devices of their own design.

Tawashi and Piccolo[19] have examined recent theories of crystal growth and dissolution, and have considered the role of substances which act as inhibitors of these *two processes*. F.D. & C. Blue No.1, at concentrations of 100 mcg./ml., reduces the dissolution rate of sulfaguanidine by 55%.[20] This finding is consistent with the theory that dye molecules are preferentially adsorbed at the primary dissolution sites on the sulfaguanidine crystal. Polyvinylpyrrolidone inhibits the crystal growth of sulfathiazole.[21] It has been proposed that the polymer forms a non-condensed, netlike film over the sulfathiazole crystal surface.

Testosterone propionate has been found to exhibit regular solution behavior only in saturated hydrocarbon solvents.[22] Its solubility is more accurately predicted as the temperature approaches the melting point, and as the molar volumes of the solvents approach that of the solute. Entropy considerations lead one to the conclusion that specific solute-solvent interactions occur in some solvents, increasing the solubility of testosterone propionate, and causing deviations from regular solution behavior. Shifts in the infrared stretching frequencies of the ketone and ester carbonyl groups are approximately proportional to the degree of deviation shown by solvents from regular solution behavior.[23]

Micellar solubilization is a technique used to increase the aqueous solubility of any hydrophobic substances. Testosterone,[24] phenols,[25] benzoic acid,[26] papaverine hydrochloride,[27] and barbiturates[28] are among compounds whose solubility has been studied in solutions containing surfactants. The dissolution rate of a solid in a micellar solution is not proportional to the solubility of the compound in the dissolution medium. From an evaluation of dissolution rate data and theories, Gibaldi and coworkers[29] have concluded that, depending upon hydrodynamic conditions, the dissolution rate of a solid in a surfactant solution will be proportional to the effective diffusion coefficient raised to a power between 0.5 (for dissolution from a static disc) and 1.0 (for dissolution from a rotating disc).

Surface Phenomena - The nature of the interactions resulting from the adsorption of drug molecules at the surface of various powders has been the subject of a number of investigations. Diffuse reflectance spectroscopy seems to be a useful tool for the detection of such interactions. Attempts have been made to correlate diffuse reflectance spectral shifts with the occurrence of physical adsorption and chemisorption,[30] charge-transfer interactions,[31] and the formation of metal chelates.[32]

In vitro rates of binding of several conjugated bile salt anions to cholestyramine have been studied at 37°, alone and in the presence of varying concentrations of sodium chloride.[33] A second-order kinetic model represents the interaction data satisfactorily. The rate constants for the adsorption process decrease in the presence of increasing concentrations of inorganic electrolyte. There is a log-log relationship between the second-order interaction rate constant and the speed of agitation. Binding of bile salt anions to cholestyramine apparently occurs by means of a diffusion-controlled process. Binding data fit the Langmuir adsorption equation, and increases in affinity constants are observed as the number of hydroxy substituents on the bile salt ring structure decreases. An increase in the fatty acid chain produces a reduction. It has been suggested that the binding mechanism consists of a primary electrostatic component, reinforced by a secondary nonelectrostatic interaction. The strength of the latter force is dependent on the degree of hydrophobicity of the adsorbate molecule.

Although bile salts have previously been found to inhibit the growth of cholesterol crystals, Mufson and Higuchi[34] have shown that they are not strongly adsorbed onto cholesterol particle surfaces. This behavior is in contrast to that of alkyl surfactants, which are strongly adsorbed. The authors assume that the relatively rigid bile salt molecules can be adsorbed only onto specific sites on the cholesterol surfaces, while the more flexible alkyl surfactants are somewhat less restrained in their interactions.

Aromatic carboxylic acids appear to be adsorbed onto the fused benzene ring plane of graphite in such a way that plane-to-plane stacking occurs between the acid molecules and the graphite structure.[35] A better correlation was found between the first Langmuir adsorption equilibrium constant and the plane size of the adsorbate

molecules than with either the pK_a of the acid, or with the Hansch-Fujita substituent constant.

The fact that many drugs exhibit surface-active properties has led to the postulate that there is a relationship between surface and biological activity. In particular, many drugs which exert their action at biological membrane surfaces, e.g. local anesthetics and the substituted phenothiazines, also exert significant surface activity at a variety of other interfaces. Frequently surface activity is a reflection of the hydrophobic characteristics of a drug molecule,[36] characteristics known to influence availability as well as reactivity at a site of action. The ability of some substituted phenothiazines to reduce the surface tension of aqueous solutions has been studied in order to evaluate their hydrophobic behavior. Zografi and Munshi[37] have observed that substitution on the phenothiazine ring enhances surface activity in the order $CF_3 >> Cl > H$. Changing the position of the chloro group on the ring significantly influences surface activity, the order being $3Cl > 2Cl > 1Cl$. The remarkable hydrophobicity of chlorpromazine derivatives is demonstrated by the fact that intrinsic partition coefficients for all derivatives, with the exception of the very polar metabolite chlorpromazine sulfoxide, range from 10^4 to 10^5.[38]

Nuclear magnetic resonance spectroscopy has been used to study self-association in promethazine hydrochloride,[39] in 2-butyl-3-benzofuranyl 4-[2-(diethylamino) ethoxy]-3,5-diiodo-phenyl ketone hydrochloride (SKF 33134A),[40] and in d-propoxyphene hydrochloride.[41] Florence[42] has measured the properties of β-diethylaminoethyl diphenylpropylacetate hydrochloride (SKF 525-A) by light scattering, surface tension, and microelectrophoretic techniques. He suggests that caution should be exercised in the interpretation of enzyme inhibition results obtained with a compound of this type since it exhibits surface activity, and surfactants are known to exert an appreciable effect on certain enzyme systems.

Perrin and Idsvoog[43] have found that optical activity can be induced into a symmetrical molecule by an optically active surfactant in micellar form. L- and D-N-decyl-N,N-dimethylalanine hydrobromides (betaines) were used as surfactants, and sulfaethidole as the optically inactive molecule. The use of optically active surfactant monomers, such as β-D-octyl glucoside, or of solubilized optically active molecules, offers the possibility of employing optical activity as a probe for studying properties of interfaces and of understanding the effect of the interface composition on the optical activity itself.[44]

Complexation - While much is known about the stoichoimetry of complexes, little is known about the specific interactions responsible for the stability of these species. There are few examples of complexes of pharmaceutical interest whose crystal structures have been elucidated. Craven and Gartland[45] have reported the crystal structure of the 2:1 complex of barbital with caffeine.

Additional information about the structure of complexes has come from investigations of solvent effects on stability constants. Kristiansen and coworkers[46] have reported that the stability constants of such complexes as those formed between riboflavin and salicylate ion (1:1), between menadione and caffeine, and between tryptophan and caffeine, decrease as the ratio of organic solvent to water increases. The complexes are much less stable in aqueous dioxane mixtures than in similar mixtures of water and the polyhydroxy compounds glycerin and sucrose. These studies indicate that water structure plays an important role in stabilizing the complexes. The net enhancement in binding as the water content in the environmental solvent increases cannot be rationalized on the basis of any single binding mechanism. Hydrophobic bonding and a nonclassical donor-acceptor mechanism may be the major forces, with the contributions from the former being somewhat less significant.

Higuchi and Kristiansen[47] have proposed that binding between organic species dissolved in water takes place most effectively between two large, distinct classes of structures, divided into Class A and Class B. Although members of Class A and B bind with others within their own class, the strongest interactions seem to be between the two groups. Typical examples of Class A are the uncharged alkylxanthines and tetramethylpyrimidopteridinetetrone. Among compounds in Class B are various benzene derivatives, salicylates and trans-cinnamic acid anions.

Kakemi and coworkers[48] have observed that complexation between two structurally dissimilar compounds is favored over that between two similar compounds. In aqueous media, for example, the extent of interaction is greater between polar and polarizable compounds than between two polar compounds or between two polarizable molecules. Water seems to be important in bringing solutes together through hydrophobic bonding, but once the molecules are in close proximity, an interaction similar to polarization bonding may become operative, and it is this bonding which stabilizes the complex.

There seems to be a reasonably good correlation between complex stability and the planar area of interactants. Cohen and Connors[49] have plotted the standard unitary free energy change for complex formation in aqueous solution against estimated maximal overlap area for fifty complexes. The dispersion of points in the plot was considered to be a second-order effect, possibly correlatable with specific structural features in substrate and ligand.

Polymorphism and Pseudopolymorphism - There is an increasing awareness of the problems associated with the maintenance of phase purity during the development and storage of pharmaceutical dosage forms. The crystalline form, habit and degree of crystallinity of a substance frequently affect such bulk properties as the ability of a powder to flow freely, and the ease with which tableting and capsule filling can be carried out. In addition, the performance of the dosage form may be affected by variations in the dissolution rate, biological availability, chemical and physical stability, suspendability and rheology which often accompany phase transformations.

Consequently, the identification and characterization of polymorphic forms and of crystal solvates of pharmaceuticals is now an important part of the protocol for the physical investigation of new drug entities.

Thermal analysis has been used to identify and characterize polymorphs of chlordiazepoxide hydrochloride,[50] phenobarbital monohydrate,[51] chloramphenicol palmitate,[52] 3-(3-hydroxy-3-methyl-butylamino)-5-methyl-as. triazino [5,6-b] indole (SKF 30097),[53] sulfathiazole,[54] and sulfanilamide-$\bar{d}_4$.[55] Solubility vs. solvent composition diagrams have been useful in the systematic study of pseudo-polymorphism in the antibiotics cephaloglycin and cephalexin.[56] This technique is recommended for the detection of solvate formation when the instability of the compound at elevated temperatures precludes the use of conventional thermal methods, or when poor crystal development limits the use of microscopic methods.

Six polymorphic forms of aspirin have been detected using differential scanning calorimetry.[57] A Kofler hot stage was used to confirm the melting points of the polymorphs and to observe solution phase transformations of pairs of polymorphs. Density differences are reported for four of the polymorphs which could be isolated, but only minor variations in X-ray diffraction patterns could be observed.

Drug Stability - The oxidation of apomorphine by molecular oxygen is apparently first order in apomorphine, does not exhibit a lag time, and is catalyzed by copper (II) and iron (II).[58] The effect of these ions is negated by the use of 0.01% sodium edetate. Apomorphine can be stored at room temperature for fifteen years or more if its solutions are prepared under nitrogen at pH 3, with sodium metabisulfite and hydrochloric acid.

A cyclic equilibrium process has been proposed as the mechanism for the hydrolysis of pilocarpine.[59] The equilibrium position depends on pH, shifting to pilocarpate at high pH, and to pilocarpine at low pH. The reaction is catalyzed by both hydrogen and hydroxide ion. Hydrolysis at high pH values is accompanied by some epimerization, but the latter reaction occurs at an appreciably slower rate.

Sulfacetamide sodium undergoes degradation in aqueous solution by two routes, oxidation and hydrolysis. Ophthalmic solutions of this compound frequently contain sodium metabisulfite as an antioxidant to decrease the rate of development of the yellow color characteristic of degraded solutions. Davies and coworkers[60] have shown that sodium metabisulfite accelerates the hydrolytic degradation of sulfacetamide to sulfanilamide, whereas sodium edetate does not influence the rate.

The availability of high speed computers has simplified the task of stability prediction based on accelerated temperature studies. Bentley[61] describes a method based on weighted least-squares analysis which can be easily adapted for computer analysis. A statistical test is presented for determining the applicability of the Arrhenius relation to the data at hand, and the usefulness of the technique is

illustrated by applying it to data for the decomposition of chloramphenicol. Maulding and Zoglio[62] have made use of nonisothermal stability studies to obtain from a single experiment the activation energy, reaction rate and stability prediction at any desired temperature. Their technique is demonstrated using data for the inversion of sucrose and the hydrolysis of ethyl acetate.

Interest in the kinetics and mechanism of organic reactions occurring in the presence of micelles has been prompted by recognized analogies between enzymatic and micellar catalysis, and between the structures of proteins and micelles. The subject of micellar effects on the rates of organic reactions has been reviewed by Fendler and Fendler.[63]

The hydrolysis of procaine hydrochloride, procaine methyl chloride and procaine ethyl chloride has been studied in an aqueous medium, in mixed aqueous systems containing polyethylene glycol 300 and 400, and in an aqueous gel consisting of 55% polyoxyethylene tridecyl ether, identified as a neat smectic system.[64] The reaction rates are considerably slower (300- to 1100-fold) in the liquid crystalline phase than in aqueous media. Rates in polyethylene glycol solutions are intermediate between those in the two other media. Reactions in the smectic phase are characterized by relatively low apparent activation energies and by large negative entropies of activation. Data obtained on spectral shifts in the ultraviolet region suggest that the esters are located within the polyoxyethylene layers of the lamellar micelle.

Flynn and Lamb[65] have reported that solvolysis of methylprednisolone-21-phosphate in dilute aqueous solution (less than 0.005 M) is qualitatively similar to that observed for the methylphosphate and other simple monoalkyl phosphates, particularly in the pH range 3-8. In more concentrated solutions (greater than 0.02 M), however, there is an acceleration of reaction velocities and marked deviation from the expected pH dependency. This change in chemical behavior is attributed to association colloid formation, and this interpretation is supported by independently determined critical micelle concentration values.

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Chapter 26. Biopharmaceutics and Pharmacokinetics

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Biopharmaceutics - The relationship between the physicochemical properties of a drug in a dosage form and the biological response observed following its administration is the subject of biopharmaceutical studies. The ability to carry out in vitro studies on the dosage form which show in vivo correlations with biological availability is a fundamental problem and has been the principal concern of a series of articles by Wagner.[1] Gibaldi and Weintraub[2,3] demonstrated an absolute quantitative correlation between the absorption and in vitro dissolution of three aspirin dosage forms utilizing a rotating-flask apparatus to determine dissolution. The advantages of this apparatus over the beaker method, where mound formation takes place, were described with a specific example relating to the effects of surfactants. Factors affecting the release and availability of aspirin from hard gelatin capsules were examined in vivo and in vitro.[4] A number of workers complexed or permitted drugs to interact with pharmacologically inert ingredients so as to increase the availability of the drug[5,6] or to prepare a sustained release dosage form[7-10] showing good in vivo-in vitro correlations. Blood pressure lowering from sustained release pentaerythritol tetranitrate tablets was found to correlate well with in vitro release measurements.[11] However, as Florence[12] points out, care must be taken in relating increases in dissolution rates in vitro to an increase in biological activity of the drug, especially when surfactants are used. A more subtle possibility of an incorrect correlation with absorption rates was noted by Chiou and Riegelman,[13] who investigated the increased oral absorption of griseofulvin in dogs following preparation of a solid dispersion of the drug in polyethylene glycol 6000. It was shown that the absorption of griseofulvin (from this preparation and from micronized solid dosage forms) was the rate-limiting step in the disposition of the drug, so that the slow rate constant in the pharmacokinetic model was, in fact, the absorption rate constant.

Physiologic factors and drug interactions may also affect the extent of drug absorption. Poole[14] noted significant differences in the blood levels and availability of monobasic penicillins (nafcillin, dicloxacillin, and penicillin) following oral absorption of these compounds in beagle dogs. The blood levels and availability of the doses in females were more than twice those found for males. These differences were not noted for amphoteric penicillins, which seemingly implicated a possible difference in GI acidity as the causitive factor, since no sex differences in elimination were noted following i.v. administration. Riegelman et al.[15] found that the amount of orally administered griseofulvin which was absorbed after phenobarbital pretreatment fell well below that seen when no phenobarbital was given, although the elimination kinetics of griseofulvin were identical with or without phenobarbital pretreatment.

Since the majority of drugs are given orally, much work has been

directed toward understanding the mechanisms of intestinal absorption and in attempting to modify the drug or dosage form so as to improve absorption. Bates and Gibaldi[16] have presented an excellent review of the GI absorption of drugs. Suzuki et al.[17,18] derived a set of theoretical models to describe the mass transfer of acidic and basic drugs across the GI tract. They considered the effects of partition coefficient, pKa, aqueous and lipid diffusion constants, thickness of the diffusion layer, and agitation rates. Winne[19] derived a kinetic model which describes the influence of intestinal blood flow on the intestinal absorption of solutes. Diamond and co-workers[20,21] found that the intestinal absorption of sulfaethidole, salicylic acid, barbital, and haloperidol decreased as mesenteric blood flow rates were decreased. The dependence of the absorption of non-dissociable substances on intestinal blood flow rates was shown to be a function of the membrane resistance to the passage of the drug.[22] Jusko et al.[23] found that the absorption of riboflavin increased with age in human subjects ranging from 0.25 to 40 years. It appears that an age-dependent increase in retention of the vitamin at intestinal absorption sites is responsible. This retention is apparently a function of decreased intestinal transit rate in older subjects. Correlations of the relative absorption of drugs as a function of partition coefficient were observed to be significant for sulfonamides[24] and insignificant for quaternary ammonium salts.[25] Doluisio and co-workers[26] reported that highly lipid soluble drugs such as the phenothiazine and butyrophenone tranquilizers gave a biexponential loss of drug from the lumen solution in the in situ rat gut preparation. In vitro experiments showed that this type of loss correlated well with a model postulating reversible transport between luminal solution and the membrane, and irreversible transport from the membrane into the blood.

The effect of the specific ions in the mucosal bathing solution was investigated by a number of workers. Mayersohn and Gibaldi[27] found that the passive transfer of drugs across the everted intestine varied as a function of specific cation concentrations and that this effect was correlated well with the fluid uptake of the membrane. Turner et al.[28] noted the apparent differences in directional permeability of drug ions across the everted rat intestine. It appeared that these differences may be explained in relation to sodium transport. More recent work by Benet and co-workers[29] identified differences in drug transfer rates and gut integrity as a function of buffer composition. Perrin and Vallner[30] showed that the absorption of tetracycline through the in vitro rat stomach at acid pH values could be influenced by the anion, and that the absorption appears to be related to the surface activity of the anion buffer solution. The enhancement of drug absorption through the GI tract by complex formation or solubilization by surfactants, including the natural bile salts, was attempted in a number of laboratories. Gibaldi and Feldman,[31] in a selected review of mechanisms of surfactant effects on drug absorption, discussed the influence of surface active agents on drug solubility and dissolution rate, gastric emptying, and membrane permeation. Special emphasis was given to the role of physiologic surfactants in the gastrointestinal absorption of drugs. As stated by Sugimoto[32] and Kakemi et al.,[33] there are at least three ways solubilization or complexation can affect drug absorption: 1) A change in absorption may be due to an absorption rate constant for the complex which

is different from that of the free drug, e.g., increased nicotine absorption due to ion pair formation[34]; enhanced prednisone absorption following complexation with N, N-dialkyamides[35]; 2) A complexing agent may affect the accumulation of the drug in the tissue or may influence the binding of the drug to the mucosa, e.g., bile salts enhance phenol red absorption[33-36] and vitamin A absorption is enhanced by polysorbate 80[37]; and 3) An agent may have a direct effect on the permeability characteristics of the mucosa, e.g., bile salts enhance sulfaguanidine absorption reversibly[38] and a variety of surfactants increased the absorption of water soluble antibiotics in the canine fundic stomach pouch.[39]

Barr and Riegelman[40,41] studied the effects of metabolism, tissue accumulation, and blood flow on the intestinal transport of salicylamide, and found that intestinal glucuronide formation is capacity-limited when lumen concentrations of salicylamide exceed 10^{-3}M. Possibly, of even greater interest was their conclusion that the rate of salicylamide glucuronide transport out of the epithelial cell rather than the rate of metabolism in the cell is the rate-limiting step for the appearance of glucuronide in the plasma. Fischer and Millburn[42] found that although C^{14}-diethylstibestrol readily entered everted rat intestinal sacs and appeared in the serosal solution as the monoglucuronide, the sacs were relatively impermeable to administered doses of the glucuronide. Schnell and Miya[43] showed that pretreatment with a carbonic anhydrase inhibitor decreased the absorption of amphetamine and increased the absorption of salicylic acid while having no effect on the absorption of urea from the rat ileum. These changes were consistent with an increased acidity in the lumen following acetazolamide pretreatment. Drug transport and availability has also been measured for rectal[44], buccal[44-46] and percutaneous absorption.[47,48]

Pharmacokinetics - While biopharmaceutics is essentially the study of the effects of the dosage form on the input (absorption) of a drug into a biological system, pharmacokinetics is concerned with the disposition of the drug once it becomes available for input. The goal of a pharmacokinetic study should be to relate a clinical response to the pharmacokinetic parameters used in describing the time course of a drug and its metabolites in the body. Very few pharmacokinetic studies have successfully related clinical or pharmacological responses to the model parameters and present efforts are aimed at determining which pharmacokinetic parameters can be related meaningfully to pathological states in the patient. The pharmacokinetics of methotrexate in mice were described by Bischoff, Dedrick, and Zaharko[49] using a flow model representing real physiological spaces. Blood and tissue levels (including lumen - the major site of concentration build-up and toxicity) could be adequately described utilizing three compartments: the lumen of the gut, the liver, and the rest of the body. Although modelling of this type appears quite different from the use of a compartment to describe each exponential needed to fit the data, the final equations from both methods appear identical. However, when used successfully, the flow model has the advantage of allowing the investigator to offer a physiologic interpretation to the parameters. Dedrick, Bischoff, and Zaharko[50] were also able to correlate methotrexate concentration in plasma with time for 5 different animal species: mouse, rat, monkey, dog,

and man, yielding a single curve for data in all five animal species. They were probably able to make this successful correlation because the drug is not metabolized significantly and since changes from animal to animal would depend only on the size of various compartments and on blood flow rates to these compartments.

Most work during the past year involved use of the more conventional compartment models which have been described in a number of books.[16,51,52] Bischoff and Dedrick[53] presented a thorough mathematical analysis of the two compartment open model for drug distribution following drug administration in any form. Rowland, Benet and Riegelman[54] presented a general solution for the pharmacokinetic parameters which describe a drug and its metabolite in a 2-compartment model. Acetylsalicylic acid data were found to be adequately described by a model in which elimination occurs solely from the central compartment. Loo and Riegelman[55] discussed the determination of pharmacokinetic rate constants following a slow I.V. infusion. This technique will be useful in the pharmacokinetic evaluation of drugs which cannot be given by a single quick bolus injection, because of potential toxicity, irritation or limited solubility. These authors utilized Laplace transform input functions in deriving their equations, a rapid and easy method for deriving pharmacokinetic equations which should find expanded use in the next year.

Papers concerning calculations necessary to make predictions in multiple-dose therapy, from the pharmacokinetic parameters of a single dose, dealt specifically with sustained-release dosage forms[56], number of doses required to be within a percentage of steady state[57], and the advantages of using Wagner's average plasma concentration term.[58] Wagner[59] pointed out that correct relative absorption rates for different formulations of a drug product can be obtained using a one compartment model even when the two compartment model is more appropriate. Levy[60] demonstrated that errors in blank corrections in the analysis of blood samples may lead to plasma curves which appear to follow multicompartment models suggesting saturation processes, even though the curvature is only a function of an analytical error. It should also be pointed out that an error in the y-axis intercept of a Beer's Law plot would have a similar effect and therefore the reviewer would suggest that in many cases it would be proper to force the Beer's Law plot through zero. These errors become significant only at very low concentrations and this most probably is the reason that so many 72 and 96 hr. data points fall below the log-linear line. Mueller and Lieberman[61] have considered the statistical significance of data selections, methods of computer data fitting, the use of means or medians for groups of data, and weighting functions in the determination of pharmacokinetic parameters. Evert and Randall[62] have introduced the use of the Continuous System Modeling Program for the solution of nonlinear equations in the evaluation of non-steady-state systems for injected drugs and for isotope dilution studies. Derivations are carried out by using the "state-space" approach of linear system theory. The methodology appears very useful but it will require a knowlege of the mathematics of state-space matrix theory before the procedures can be understood.

The effects of protein binding on the disposition of drugs has been considered in both theoretical[63,64] and experimental[65-67] treatments. Kakemi et al.[68] showed that the biological half-lives of sulfonamides were decreased when a strongly protein bound anti-inflammatory agent, 5-n-butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine was administered simultaneously. O'Reilly and Levy[69] noted that simultaneous administration of warfarin and phenylbutazone potentiated the pharmacologic action of warfarin even though elimination was enhanced. Both effects are attributed to the fact that phenylbutazone competitively displaces warfarin from protein binding sites in plasma and tissues, allowing free drug concentrations in the liver to be raised. Renal excretion studies by Jusko et al. showed that riboflavin elimination involves active tubular secretion[70,71] and saturable tubular reabsorption[72] in both dogs and humans. It is interesting to note that although the authors propose a model involving nonlinear processes the plasma and urinary excretion rate data are well fit by a triexponential equation suggesting a linear three compartment model. Probenecid appears to inhibit both the tubular secretion and the specialized reabsorption of riboflavin, while also decreasing by about one-half the apparent volume of distribution.[70] However, probenecid had no effect on riboflavin elimination during hemodialysis of two functionally anephric patients,[73] suggesting that the volume of distribution change in normal patients might be only an indirect result of its effect on the renal excretion of the vitamin. Similarly Gibaldi et al.[74] showed that although probenecid markedly decreased the mass transfer rate constant for elimination of penicillin from the central compartment, it also significantly increases the fraction of drug in the volume of distribution from which elimination occurs. Renal clearance studies were also reported comparing: the rate of active tubular secretion for mandelic acid and its homologs[75,76]; the effect of varying grades of renal insufficiency on thiamphenicol[77] and doxycycline[78]; and the effect of urinary pH on sulfonamide clearances.[79,80]

The effects of biliary excretion on drug kinetics is receiving increasing attention in pharmacokinetic studies.[81-85] Klaassen[86] showed that the increase in biliary flow and excretion following phenobarbital treatment enhances the plasma disappearance of a number of drugs. Nogami et al.[87] found a dose dependent relation for the biliary excretion of riboflavin. When high doses (1-10 micromoles) of the vitamin were administered, rapid biliary excretion occurred instantaneously with 80% of the dose being excreted in 2 hr., followed by a low rate constant for excretion in the bile which was similar to those values observed for low doses (0.05 - 0.1 micromoles). This type of dose dependency was also noted for five azo dyes.[88] The number and positions of sulfonate groups affected the relative biliary excretion of the dyes.

VonBahr and co-workers[89] found a good correlation between in vivo half-lives for phenylbutazone, antipyrine, and oxotremorine, all compounds with small volumes of distribution in vivo, and values found in an isolated perfused liver. The in vivo half-lives for nortriptyline and desmethylimipramine, compounds with large volumes of distribution, were 15 to 50 times longer than those obtained in the perfused liver. This anomaly

however, might not have occurred if the authors had considered *in vivo* and *in vitro* clearances instead of half-lives. A good linear relation between *in vivo* and *in vitro* rate constants was found for phenylbutazone following stimulation of microsomal enzymes by phenobarbital.[90] The effect of metabolic enzyme induction was also studied for the following drug-inducer pairs: thyroxine-diphenylhydantoin,[91] triiodothyronine and thyroxine-phenobarbital,[92] thiopental-SKF 525A,[93] warfarin-heptabarbital;[94] bishydroxycoumarin-heptabarbital.[95] Although phenobarbital has been shown to stimulate the metabolism of diphenylhydantoin in single dose studies, long term therapy showed no significant stimulation.[96] Arnold and Gerber[97] found that the rate of disappearance of diphenylhydantoin in 70 adults was often not a first order process and that dose dependent increases in rate occurred in some patients. Shand, *et al.*[98] compared plasma propranolol levels following oral and I.V. administration and found the "availability" of the oral dose to be much lower, probably due to first passage metabolism. Similar oral-I.V. availability differences due to metabolism were seen for lidocaine[99] and pentazocine.[100] A study of the kinetics of metabolism and the urinary excretion of the optical isomers of mandelic acid revealed no differences in urinary excretion, however, the L-(+) isomer was metabolized approximately twice as fast as the D-(-) isomer.[101] Levine and Dizon[102] presented evidence for capacity-limited biotransformation of sulfanilamide. The distribution, metabolism, and excretion in rats of moperone[103] showed this new neuroleptic drug to undergo rapid hepatic metabolism, as opposed to its prototype compound haloperidol and another congener, trifluperidol.[104]

During the past year more than 80 papers have appeared describing the pharmacokinetics-the absorption, distribution, and metabolism of various drugs. Although many of these papers cannot be covered in this limited review, the importance of these studies should not be minimized. There has been a lack of valid, comprehensive data, and it is ironic that the half-lives of some frequently used drugs are unknown or are suspect because of inaccuracies inherent in measuring small concentrations in the blood. Ritschel[105] has reviewed the importance of biological half-lives of drugs and commented on the physiological and pathological conditions which may affect the half-life. He has prepared a table of the half-lives of over 200 drugs. However, no critical evaluation of the literature values is made and in some cases more recent references have been by-passed, resulting, in a few cases, in values that are inaccurate (e.g., aspirin 2.5-5.8 hrs. as opposed to the more widely accepted value of 15 minutes; the higher values are closer to the half-lives for salicylate). Kaplan *et al.*[106] derived a single pharmacokinetic model for chlordiazepoxide hydrochloride and its N-demethyl and lactam metabolites. Two compartment parameters were determined for all three compounds in this excellent work. Kaplan[107] has also examined the pharmacokinetics of Coumermycin A_1 in humans following I.V. and oral administration. Although only four subjects were studied, it appears that dose-dependent kinetics may be exhibited by this drug as evidenced by decreased elimination rate with an increase of dose on a mg./kg. scale. Kaplan attempts to differentiate between two alternative 2-compartment models, one with elimination from the central compartment and the other with elimination from the peripheral compartment. Rowland

et al.[54] pointed out that this approach is unsound unless blood levels of the metabolite are monitored also. The small differences noted by Kaplan[107] for absorption rate and % of dose absorbed at various times must be the result of calculation errors due to approximations, since there should be no model-dependent differences for any value describing absorption.

Smith and Haber[108] related digoxin intoxication to serum concentrations of the drug and showed that a clinically meaningful relationship exists between serum digoxin concentrations and disturbances of rhythm in patients with cardiac disease. Jelliffe and co-workers[109] suggested an improved method of digitoxin therapy, where daily maintenance doses are determined with respect to renal function measurements and the average rate of metabolic conversion of digitoxin to digoxin. The possibility of predicting the action of a newly synthesized drug from its structure and physicochemical properties has been discussed by Raaflaub[110] and by Seydel and Wempe.[111] The latter authors report, in abstract form, having established a relationship between physicochemical parameters and pharmacokinetic properties for substituted 2-aminopyridines which allowed them to select 2-sulfa-pyridines with certain pharmacokinetic properties. These compounds were synthesized and found to have the properties and antibacterial activity predicted.

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Chapter 27 · Alkaloids and Other Natural Products

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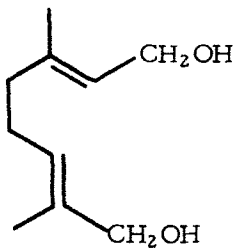
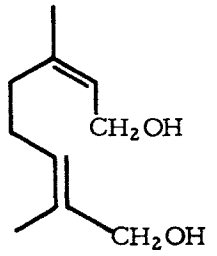
The task of searching the alkaloid literature was made easier this year with the appearance of two volumes that listed alkaloid-yielding plants and their respective alkaloids. The one by Willaman and Li[1] is an updating (1957-1968) of the well-known, but now out of print, book by Willaman and Shubert, while the other[2] is the result of a computer-assisted reduction of the literature through mid-1968. Volume XII of Manske's "The Alkaloids" appeared with approximately one third of the book being devoted to the diterpene alkaloids. Other sections include Alstonia, Senecio, and Papaveraceae alkaloids with an additional chapter concerning the forensic chemistry of alkaloids. The chemistry and biology of the peyote alkaloids were reviewed[4] and a one-volume work[5] covering all the main classes of alkaloids was also published.

The biogenesis of the terpene portion of the indole alkaloids has been defined in greater detail and a review[6] of earlier work appeared. Tracer studies with Vinca rosea demonstrated that 10-hydroxygeraniol (1) and 10-hydroxynerol (2) will function as effective precursors to loganin, indicating that 10-hydroxylation is a primary step in the conversion of geraniol and nerol into loganin and the indole alkaloids.[7,8] The presence of deoxyloganin in Menyanthes trifoliata and V. rosea was established through dilution analysis.[9]



1



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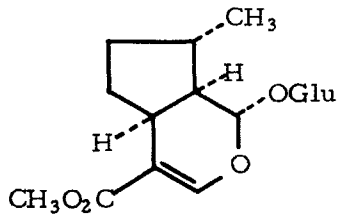
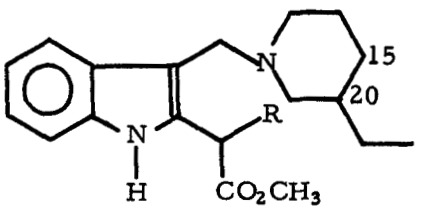


3

Three new Rhazya alkaloids possessing the general structure of a hypothetical intermediate which could bridge the major types of indole alkaloids have been obtained from R. stricta and R. orientalis: tetrahydro-(4) and dihydrosecodine (5) from the first species,[10] and tetrahydrosecodine (by dilution analysis)[11] and tetrahydrosecodin-17-ol (6) from the second.

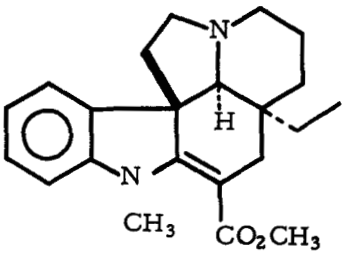


4 R = $-CH_3$

5 R = $-CH_3$
C15-C20 unsaturation

6 R = $-CH_2OH$

The biogenetically modeled synthesis of minovine[12] (7) and ajmaline[13] (8) has been reported. Both velbanamine (10), a degradation product of the antitumor alkaloids vinblastine and vincristine, and catharanthine were constructed from the same lactam intermediate (9).[14] In a series of papers[15-19] the application of transannular cyclization reactions to the synthesis of indole and dihydroindole alkaloids was described.



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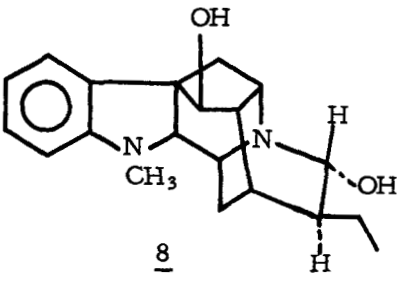

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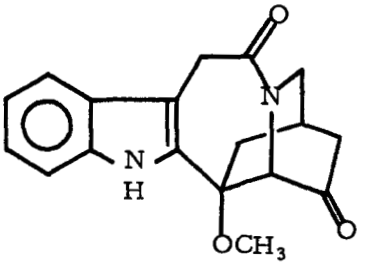
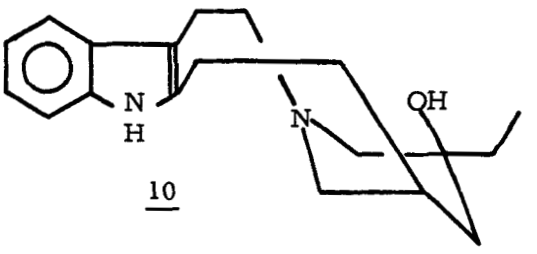


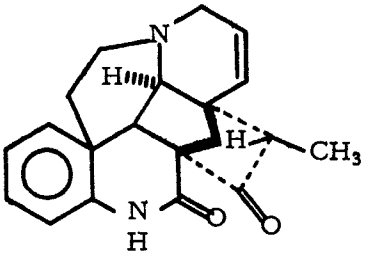
9

Catharanthine



10

The antimalaria drug quinine was synthesized by two different routes.[20,21] Meloscandonine, an alkaloid from Melodinus scandens, has been assigned structure 11.[22] Labeling experiments with Cinchona ledgeriana have helped to enumerate the middle and latter stages in the biosynthesis of the cinchona alkaloids (i.e., 12 ⟶ 15).[23,24]



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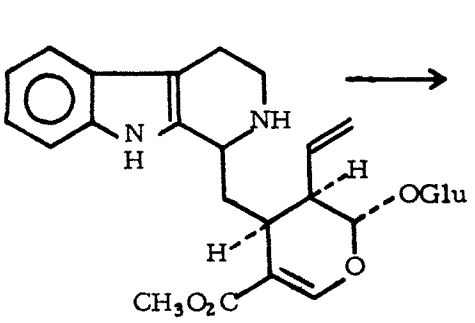

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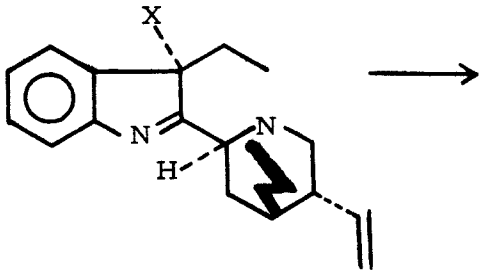

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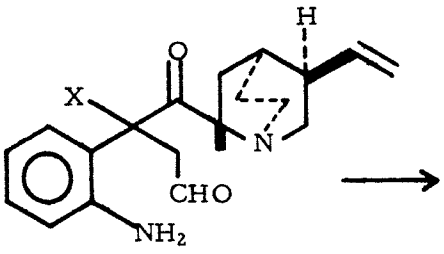

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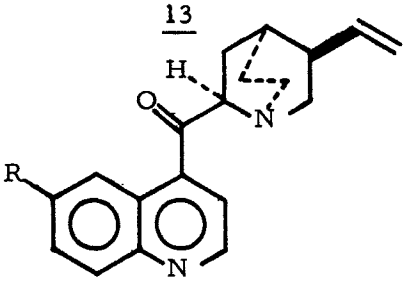
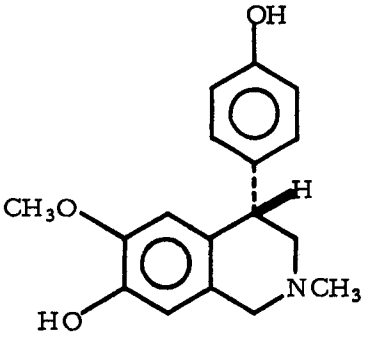


15

⟶ Quinine

Cherylline, an unusual tetrahydroisoquinoline alkaloid, was isolated from Crinum powellii.[25] Structure 16 was assigned to it from spectral and degradative evidence and was confirmed by an unambiguous synthesis.[26] The structures of two unnamed pseudoprotoberberine alkaloids from Papaver orientale have been assigned as 17 and 18.[27] Cancentrine, from Dicentra canadensis, is a new type of dimeric benzylisoquinoline

alkaloid (a morphine and cularine combination) whose structure has been established as 19[28] by physical and chemical methods.



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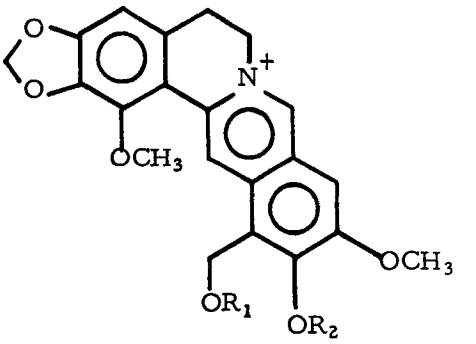
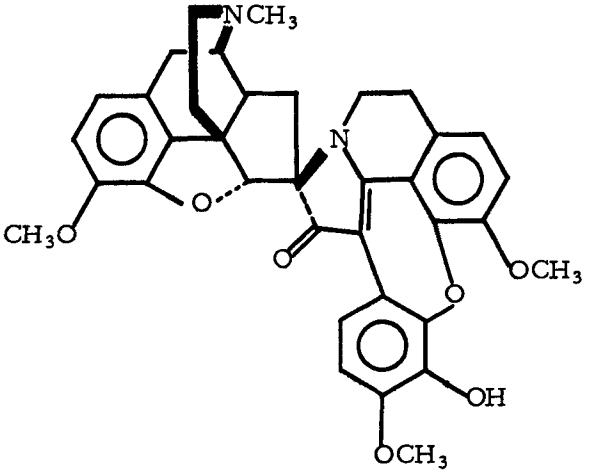


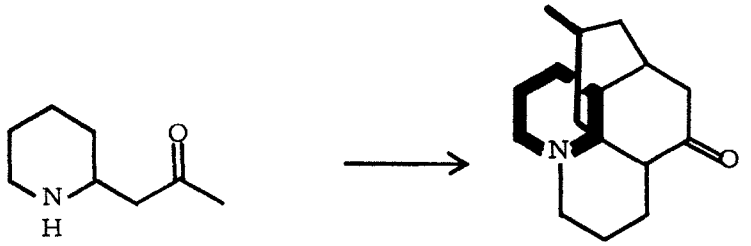
17 R_1 H; R_2 = CH_3

18 R_1 = R_2 = -CH_2-



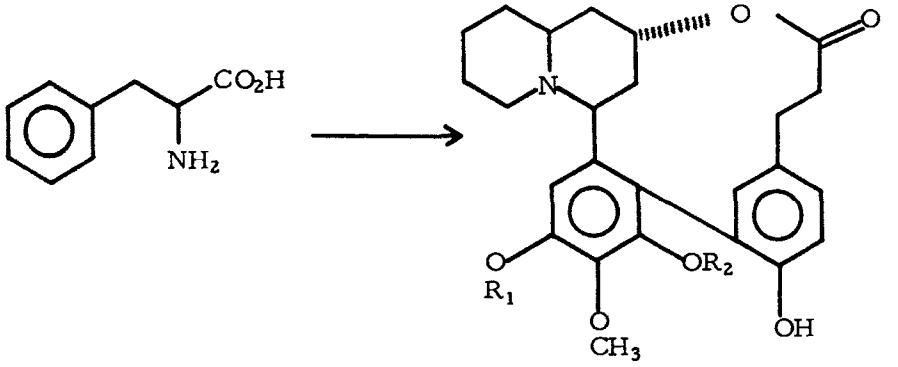
19

The full paper on the biosynthesis of the lycopodium alkaloids from lysine was published.[29] The possibility that two identical pelletierine units (20), derived from lysine and acetate, would combine to form lycopodine (21) was not corroborated by double labeling experiments with [4,5-$^{3}H_2$-2-^{14}C]-pelletierine.[30] Only the portion of the molecule drawn in heavy line was derived from pelletierine.



20 21

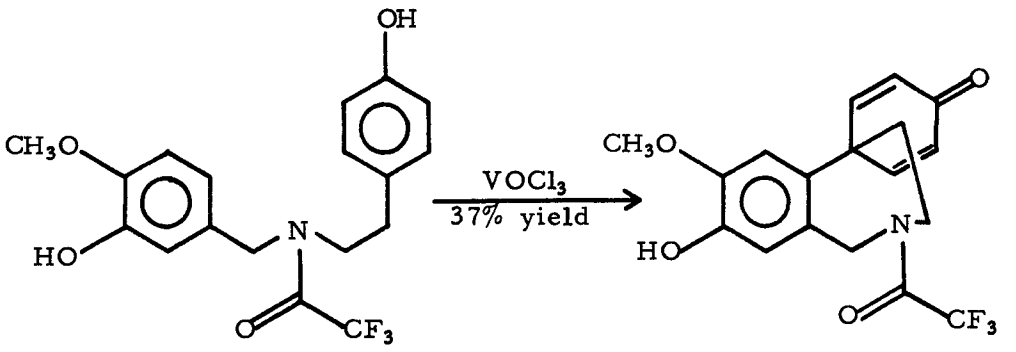
Partial degradation of the lythraceae alkaloids decodine (22) and decimine (23), isolated from Decodon verticillatus after being fed either 2-^{14}C or 6-^{14}C-lysine, indicated that the amino acid enters the alkaloid in a non-random fashion through a symmetrical intermediate.[31] It was also demonstrated that two fragments derived from phenylalanine are incorporated into decodine.[32]



22 R_1 H; R_2 OH

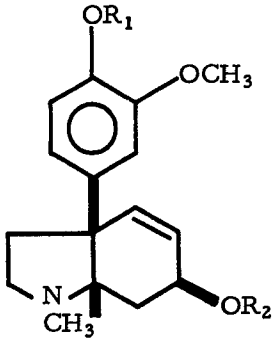
23 R_1 OCH_3; R_2 H

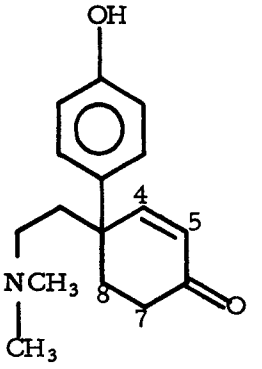
The amaryllidaceae alkaloid maritidine was synthesized by a route which featured an improved method of achieving phenol oxidative coupling (24 ⟶ 25).[33]



24 25

The small group of mesembrine alkaloids has been nearly doubled by the isolation of four from Sceletium strictum[34]: mesembrenol (26); 0-acetoxymesembrenol (27); 4'-0-demethylmesembrenol (28); and 4'-0-demethylmesembranol 29. Three seco-alkaloids: joubertiamine (30); dihydrojoubertiamine (31); and dehydrojoubertiamine (32) were isolated from S. joubertii.[35]





26 R_1 = CH_3; R_2 = H

27 R_1 = CH_3; R_2 = OHc

28 R_1 = R_2 = H

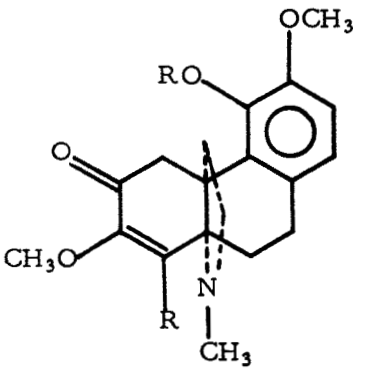
29 R_1 = R_2 = H
(double bond reduced)

30

31 C_4-C_5 double bond reduced

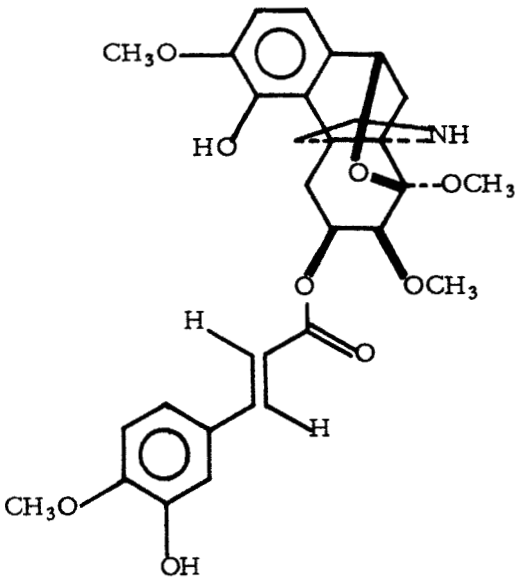
32 C_7-C_8 unsaturation

The first synthesis of hasubanonine[36] (33) and a second of the related alkaloid cepharamine (34)[37] (via the newly developed enamine annelation approach) were accomplished. Two new hasubanonine alkaloids, stephisoferuline (35)[38] and stephavanine (36)[39] were isolated from Stephania hemandifolia and S. abyssniea, respectively. Stephavanine is the most highly oxygenated hasubanonine alkaloid isolated to date.

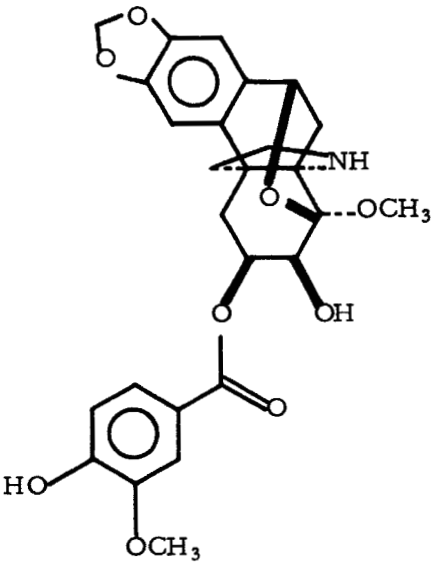


33 R = CH_3

34 R = H

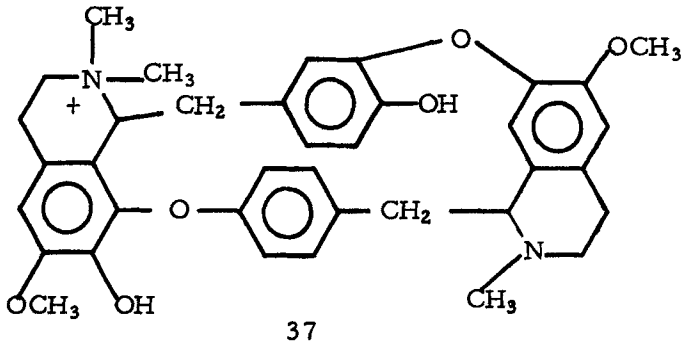


35



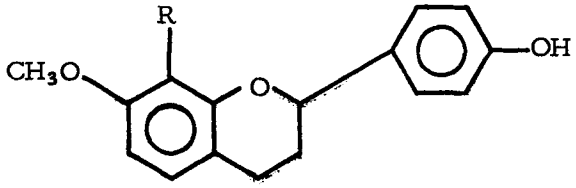
36

The accepted structures for (+)-tubocurarine chloride and (+)-chondrocurine have been found to be incorrect.[40] The former is the monoquaternary salt (37) rather than the previously proposed di-quaternary salt, and the latter its corresponding tertiary base. The substitution pattern in the A-ring is reversed from the old structure in the case of (+)-chondrocurine.



37

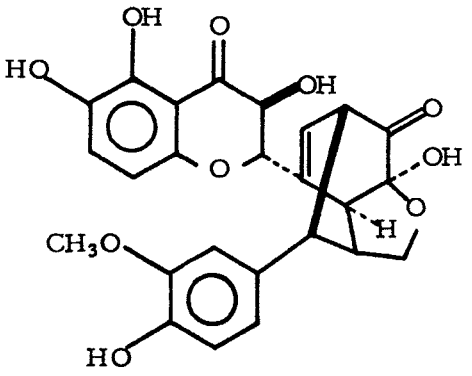
Representatives of the long sought optically-active flavans, (-)-4'-hydroxy-7-methoxyflavan (38) and (-)-4'-hydroxy-7-methoxy-8-methyl-flavan (39), were obtained from species of Dianellinae.[41]



38 R = H

39 R = CH_3

X-ray analysis has established the structure of silydianin, from Silybum marianum, as 40. It is isomeric with the antihepatotoxic agent silymarin and represents a unique combination of a dihydroflavonol and a phenylpropane.[42]



40

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Chapter 28. Reactions of Interest in Medicinal Chemistry

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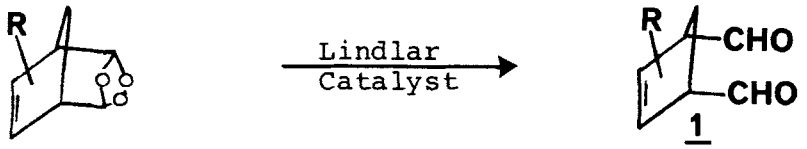
A wealth of synthetically useful research was reported in 1970. An attempt has been made here to select reactions of broad applicability; therefore, specialized fields such as peptide synthesis, have been arbitrarily excluded.

Oxidations-The use of a two phase ether-aqueous chromic acid system for oxidation of secondary alcohols to ketones has been reported to afford, in a rapid fashion, ketones which are remarkably free of isomerized and side products.[1] Amine oxides can be prepared in 90% yield from the corresponding amines, using m-chloroperbenzoic acid in chloroform, followed by purification of the reaction mixture on an alumina column.[2] A complex of hexafluoroacetone and H_2O_2 exhibits properties similar to those of trifluoroperacetic acid.[3] Using this reagent, the Baeyer-Villager reaction was executed in yields of 40-73%. The Lindlar catalyst has been used to obtain the dialdehyde 1 from several ozonides resulting from treatment of the corresponding norbornadienes with ozone.[4] The protected α-diketone 2 can be cleaved via the dithiane 3 in a way which



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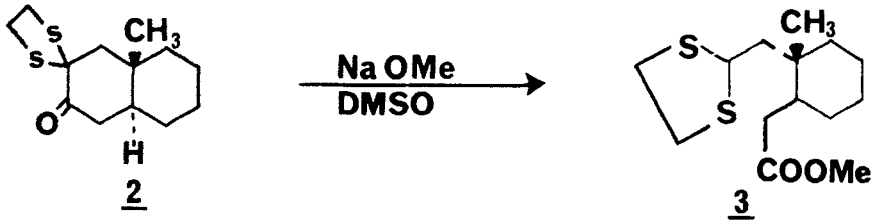
differentiates the two ends of the cleaved bond.[5] Amides and lactams can be converted to the corresponding imides in



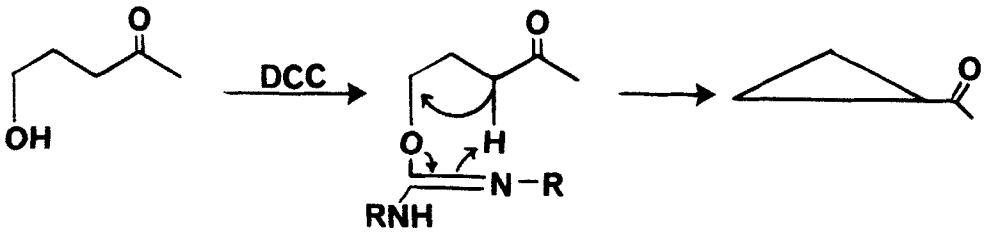
2 3

23-100% yield by the action of peracids or hydroperoxides in the presence of Mn^{+2} or Mn^{+3}.[6] Methods for facile conversion of aldehydes to nitriles involve photolysis of aldehyde-derived diphenylhydrazones which afford nitriles in 40-75% yield[7];

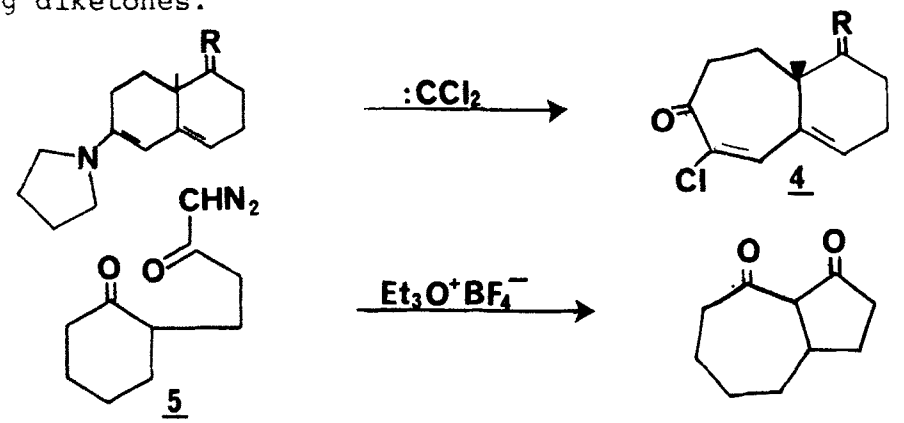
the use of $CO(NH_3)_6[Co(CO)_4]_2$ followed by Br_2 effects similar transformations.[8]

Reductions-Electrolysis has been reported to effect the selective hydrogenolysis of aryl bromide moieties in the presence of other reducible groups.[9] The yield was 94%, compared to 13% with Et_3SnH. Amides may also be reduced electrochemically on a 0.05 mole scale to the corresponding aldehydes and alcohols, depending on conditions, in 50-97% yield.[10] Facile, high-yield reduction of epoxides to alcohols has been reported to be effected without rearrangment by lithium in ethylenediamine.[11] Lithium in methylamine was reported to reduce carboxylic acids to aldehydes or amines, depending on workup, in 50-70% yield.[12] The selective reducing capabilities of disiamylborane have been extensively investigated.[13] Lithium perhydro-9B-boraphenalylhydride, an active agent which reduces ketones 94-97% stereoselectively to the corresponding steric approach control-predicted alcohols, has been described.[14] Organoboranes have been shown to add to α,β acetylenic[15] and α,β-unsaturated[16] ketones to yield α,β unsaturated and dialkyl ketones, respectively. The reactions require O_2, and apparently display free radical character. Two interesting additions of organoaluminum compounds to alkynes to afford various olefins, sometimes stereoselectively, have been reported.[17,18] Decarbonylation of aldehydes with 90-100% retention of configuration is possible, using Rhodium complexes, such as $(Ph_3P)_3RhCl$.[19] Silyl and stannyl hydrides effect high yield reduction of aryl diazonium salts, and are compatible with a wider range of solvents than is H_3PO_2.[20] NaH prepared in situ has been found to be much more active than the commercial product.[21] Using the more active NaH, hydrogenolysis of benzylic halides is possible. Sodium borohydride has been reported to reduce nitriles to amines if Raney nickel is used as catalyst.[22] The properties of P-1 nickel boride have also been further investigated.[23] This hydrogenation catalyst has been found to be more active and to cause less migration of double bonds than Raney nickel.

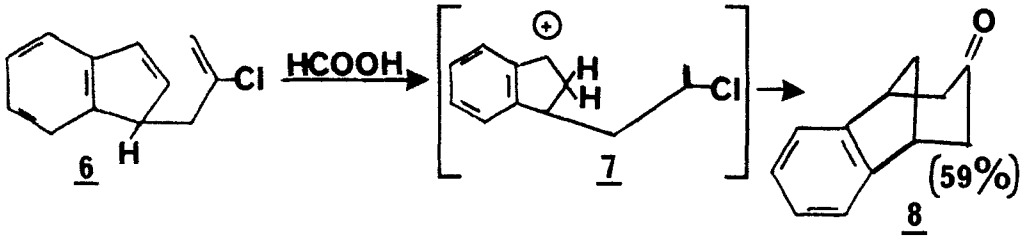
Carbocyclic Ring Formation-A mixture of Zn dust and a cuprous halide apparently functions as efficiently as a Zn-Cu couple in the Simmons-Smith procedure, and is more convenient.[24] The high yield, preparative scale electrochemical synthesis of phenylcyclopropane and cyclopropanol from the corresponding 1,3-dibromides has been reported.[25] DCC has also been shown to effect ring closure to cyclopropanes in 80% yield, in the manner shown below.[26]



An interesting ring expansion of cyclic ketones via reaction of the corresponding eneamines with dichlorocarbene has been observed.[27] In this way, ketone 4 was obtained in good yield. Also, triethyloxonium salts effect ring expansion in diazoketones such as 5 to afford the corresponding diketones.[28]

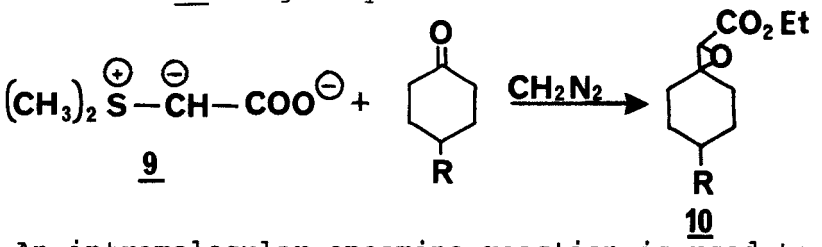


A versatile general approach to cycloalkeneones has been presented[29]: the bicyclic chloride 6, obtained from indenyl Grignard and 2,3-dichloropropene, formed the predicted carbonium ion 7, and then the ketone 8, without any rearranged products.



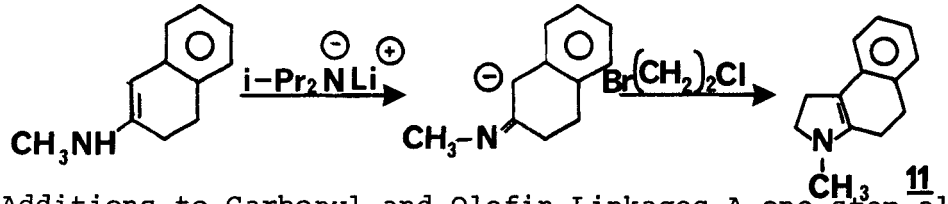
Heterocyclic Ring Formation-H_2O_2, in the presence of isocyanate as co-reactant, yields epoxides from olefins in 50-75% yield.[30] This system is advantageous when reactions must be conducted in neutral medium. 1,2-Ditosylates afford

olefins in 60% yield upon electrolysis.[31] The thietin anion 9, a new sulfur ylid, has been shown to afford the glycidic ester 10 in good yield.[32]



9 10

An intramolecular eneamine reaction is used to good advantage in the construction of 11.[33]



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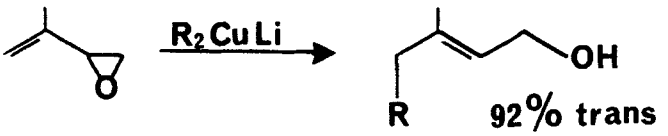
Additions to Carbonyl and Olefin Linkages-A one-step alternative to the Grignard reaction has been described, in which a mixture of the carbonyl compound and the alkyl bromide is added to a suspension of Li in THF below 0°.[35] Yields are claimed to be superior to the Grignard procedure. The Reformatsky reaction occurs in improved yield if trimethyl borate-THF is used as solvent.[36] The aldol condensation is improved if organocalcium alkyls are used as basic catalysts.[37] Mesityl oxide is thus obtained from acetone in 95% yield.

Olefins have been obtained in a stereoselective manner via the Wittig procedure.[38,39] The ylid-carbonyl adduct is treated with a strong base and an electrophile (MeI, PhCHO) to form olefins of predictable stereochemistry. The Wittig reaction has also been reported to occur with phosphonium fluorides in the absence of base.[40]

Anti-Markownikov hydrohalogenation is obtained in 75% yield by treating the olefin with an organoborane, effecting transmetallation with HgO, and treating the resulting organomercurial with Br_2.[41] Intermediates need not be purified. The solvomercuration-demercuration procedure effects Markownikov hydration of olefins in 77-98% yield.[42]

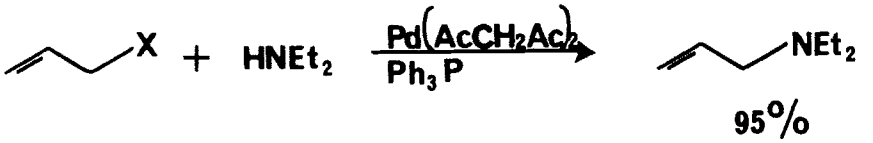
Organometallic Reagents-The usefulness of alkylcopper lithium reagents has been extended by the observation that they can replace organocadmium reagents in ketone syntheses,[43] and will

react with very hindered α-bromo ketones to effect high yield alkylation.[44] In addition, these reagents reduce oxiranes in the presence of carboxyl groups,[45] and react with allylic oxiranes in the stereoselective manner shown.[46]

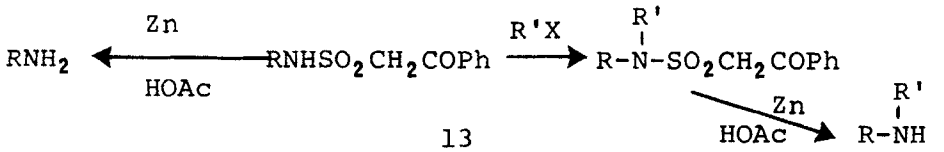


Some orientation control has been achieved in electrophilic aromatic substitution reactions effected with thallium (III) trifluoroacetate,[47] and methods for synthesis of aryl cyanides and phenols using this useful reagent have been developed.[48]

An interesting and apparently versatile allyl transfer reaction under the influence of a palladium-acetylacetone complex has been reported.[49] In addition to the N-alkylation shown below, C-alkylation at activated methylene carbon is possible.

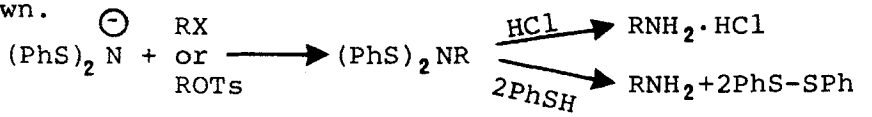


Use and Removal of Protective Groups-The use of the phenacylsulfamyl moiety as a protective group for amine functions and in the synthesis of pure secondary amines has been reported.[50] The derivative 13, obtained from an amine and



13

phenacylsulfamyl chloride may either be alkylated to yield pure secondary amine, or treated with Zn to regenerate the original amine. A versatile alternative to the Gabriel synthesis has also been devised which avoids the vigorous hydrolytic conditions associated with this prodecure.[51] In the new procedure, the bis-benzenesulfenimide anion 14 is used. Hydrolysis is then possible by either of the methods shown.



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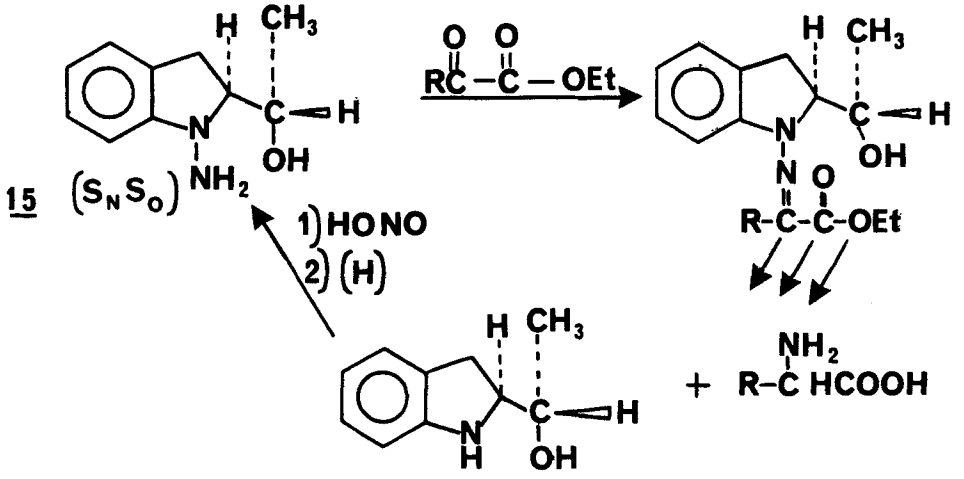
The phenacyl group may also be used to protect acids and phenols.[52] Removal is accomplished with Zn/HOAc. Hydrolysis of some methyl esters is surprisingly difficult. The use of lithium thiopropoxide in hexamethyl phosphoramide appears to solve this problem.[53]

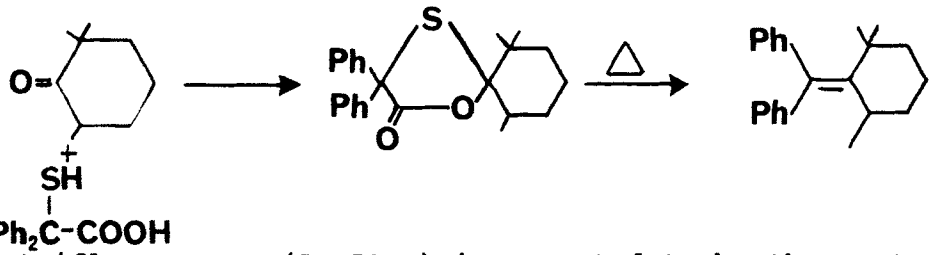
Prospects for protection of hydroxyl groups as methyl ethers were enhanced by the report that an optically active γ-hydroxy acid could be converted to the corresponding methyl ether. Following further manipulations, the methyl ether was cleaved in 77% yield by $NaBH_4$-I_2 in MeOH-CCl_4 to yield unracemized product.[54] Aryl methyl ethers are reported to be cleaved by thioethoxide ion in DMF to yield the corresponding phenol in 94-98% yield.[55] Benzoyl cyanide effects benzoylation of sugar hydroxyl groups at low temperature and in the absence of catalyst.[56]

Two methods for conversion of oximes to the corresponding carbonyl compounds, not always an easy procedure, have been developed. In the first, chromous acetate is used as catalyst,[57] and in the second $TiCl_3$ in MeOH-H_2O with acetate buffer yields the intermediate imine if this is stable to the reaction conditions, and the carbonyl compound if the imine is not stable.[58] Aldehydes are also reported to be obtainable in 80-90% yield from the corresponding dithianes if hydrolysis is carried out in THF using a HgOAc-BF_3•Et_2O catalyst.[59]

Miscellany-A most ingenious stereospecific synthesis of α-amino acids involves reaction of an α-keto ester with the chiral reagent 15.[60] Following several steps, the amino acid is obtained in 92-97% optical purity, and the chiral reagent is easily regenerated.



Several convenient olefin syntheses have been reported. N-Carbalkoxysulfamate esters undergo elimination to olefins at room temperature.[61] Epoxides afford olefins by reductive elimination under the influence of $MgBr_2$ and magnesium amalgam.[62] A twofold extrusion process for the construction of complex olefins has also been described.[63] In the example shown, the highly hindered olefin shown was obtained in 80% yield.



The triflate group (CF_3SO_3-) is reported to be the most active leaving group of the common sulfonate esters. This moiety is conveniently introduced using trifluoromethanesulfonylimidazole, a stable, low boiling liquid.[64]

Aniline is converted into benzyne in one step by pentyl nitrite in the presence of acetic anhydride,[65] with yields estimated at 10-32%.

By treating trimethylamine oxide with trifluoroacetic anhydride, it was possible to isloate 16, a typical anhydro base Mannich reaction intermediate.[66] This substance underwent the Mannich reaction with steroidal ketones in

$$(CH_3)_3N\rightarrow O \xrightarrow{(CF_3CO)_2O} (CH_3)_2\overset{\oplus}{N}=CH_2$$

16

excellent yield. Ethylene glycol solvent was also reported to improve high-temperature Mannich reaction yields, whereas DMF gave anomalous products.[67]

Several improvements in acylation techniques were announced. Butyllithium wwas determined to be superior to sodium amide in preparation of amide ions for ammonolysis reactions with esters.[68] Phosgene is more reactive than ethyl chloroformate toward eneamines.[69] The intermediate acyl chlorides may then be converted to a variety of products. α-Acetylenic aldehydes are easily prepared by the action of acetylenic Grignard reagents upon ethyl formate.[70]

Diazoketones derived from acids whose acid chlorides are inaccessible may be prepared by the action of diazomethane on

the acid in the presence of DCC.[71] Diazoketones of α-acyl-amino acids are obtainable via a mixed anhydride of the amino acid, which reacts with diazomethane to yield the diazoketone.[72]

α-Bromo acid chlorides result when acyl chlorides are allowed to react with NBS in aqueous HBr.[73]

The use of a wire mesh 0.4-0.6mm below the cold finger in a subliming apparatus makes retrieval of the purified material much easier.[74]

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