LIST OF CONTRIBUTORS

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PREFACE

This third annual volume seeks to maintain the original aims of the series: namely, to present critically and briefly new and significant contributions in various fields of medicinal chemistry which appeared in the literature during the past year, and to suggest directions in which future advances may occur. In line with these aims, it is appropriate that each new volume differ from previous ones by changes in subjects discussed and in viewpoints presented. The present volume contains many changes: one new section editor, a number of new authors, different chapter titles, additions and deletions of topics discussed, and the inclusion of the Second Medicinal Chemistry Award Address.

The names of contributors are familiar to most workers in the field of Medicinal Chemistry as those of highly productive scientists. The time they have devoted to writing and editing represent "extra" contributions which are very much appreciated.

Many favorable comments and constructive criticisms on the series have been received. More of these will be gratefully received.

Fort Washington, Pennsylvania
June, 1968

Cornelius K. Cain

AWARD ADDRESS

Biological Activity: A Medicinal Chemist's View

Sydney Archer, Associate Director of Research
Sterling-Winthrop Research Institute, Rensselaer, N. Y.

Delivered on the occasion of the presentation of the Second Award in Medicinal Chemistry at the Eleventh National Medicinal Chemistry Symposium of the American Chemical Society.
Laval University, Quebec, Canada
June 25, 1968

Mr. Chairman, Dr. Suter, ladies and gentlemen, let me take a moment to say how deeply grateful and honored I am to be designated the recipient of the 1968 Award in Medicinal Chemistry. I would like to thank my friends who nominated me and the committee who selected me for this great honor. I would also like to thank my present and former associates at Sterling-Winthrop Research Institute whose contributions have made this accolade possible. I have appended a list of my co-authors with whom I have been privileged to publish papers on medicinal chemistry and allied subjects over the past quarter century. To all my benefactors, known and unknown, I want to express my heartfelt thanks.

Most of us here this evening are vitally interested in the effect of chemical structure on biological activity, and being chemists, we know something about chemical structure. But what is biological activity? I shall not attempt to answer this question directly, but I will draw from my own experience to try to show how vitally important it is for those engaged in finding clinically useful drugs to appreciate the nature of biological activity.

What do we mean when we say that a compound is active? First, it is implicitly understood that properly controlled experiments were carried out and that certain biostatistical requirements were fulfilled. If the assay experiments involve cell-free systems, then biological effects will depend primarily on the relative affinity and intrinsic activity of the test drug. As soon as a living organism is used, a permeability factor comes into play, for example, in cell-free systems chloramphenicol and its acetyl analog inhibit protein synthesis whereas in living systems it is only the antibiotic which is active. Differences in permeability between the drugs have been invoked to explain the results. When one turns to mammalian systems a complex of other factors must be reckoned with. In the case of orally administered drugs biological activity may be affected by one or more of the

following: biopharmaceutic availability, transformation in the gastrointestinal tract, absorption from the gut, binding to blood components and other tissues, transport to and uptake by target organs, rate of excretion, enzymatic destruction and possible enzymatic activation. There are probably other factors which have been overlooked, but the list is impressive enough to make one wonder how drugs ever are successfully developed. Finally, an indirect but nevertheless extremely important factor is the relevance of the laboratory models to the desired clinical activity. Our work in the area of non-narcotic analgesics illustrates how important this aspect of biological activity really is.

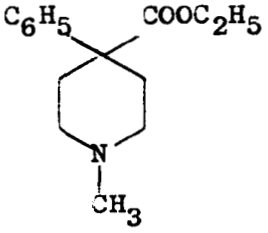
Ever since morphine had been found to be addicting, efforts have been made to find a strong analgesic of comparable potency devoid of its addicting properties. Among the earlier agents to be seriously proposed as a solution to this problem was heroin. Despite many further trials with other agents by the mid-1950's success was still to be achieved. Much knowledge had been gained: the techniques of clinical evaluation were improved, quantitation of drug-dependence was perfected at Lexington and analgesic assays in rodents were developed to the point where quantitative as well as qualitative prediction of clinical activity was extremely accurate. Most laboratories settled on some variant of Eddy's mouse hotplate test or the D'Amour-Smith rat tail flick procedure.

Nathan Eddy performed many invaluable services for the segment of the scientific community interested in this problem. For example, he and his colleagues published a series of papers in 1954-1956 in the Bulletin of the World Health Organization which reviewed critically the state of laboratory and clinical knowledge of analgesics up to that time. These authors suggested that the following structural features are necessary to produce morphine-like analgesia:

1. The presence of a central carbon atom bearing no hydrogen.
2. An aromatic ring must be attached to this carbon atom.
3. A basic nitrogen atom must be the terminus of a short chain, preferably of two carbon atoms attached to the central carbon atom.
4. The nitrogen atom must be substituted with small alkyl groups.

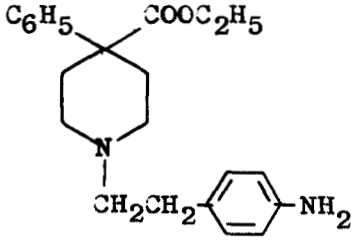
Shortly after these papers appeared, Everette May and Eddy himself discovered that replacement of the methyl group in meperidine (I) by a phenethyl group enhanced analgesic activity. Similar observations were independently in our laboratory by Grumbach and Elpern and by a Merck

group. These observations culminated in the introduction of anileridine (II) and piminodine (III) into clinical medicine.



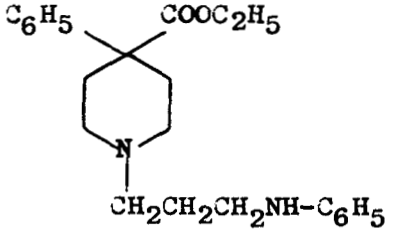
Meperidine

I



Anileridine

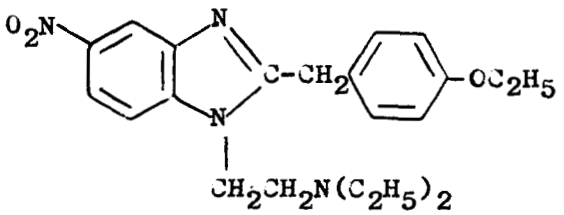
II



Piminodine

III

These newer meperidine derivatives violated the fourth of Eddy's postulates, but despite this refreshing structural departure, the compounds were able to substitute for morphine in dependent addicts. Other structural violations followed, culminating in Etonitazene® (IV) which managed to circumvent just about all of the postulated requirements yet proved to



IV

be several hundred times as potent as morphine -- and at equi-analgesic doses, just as addicting.

From the pharmacological and clinical side, little progress had been made, except that it was much easier to determine clinical activity and physical dependence liability. However, in 1954, Beecher and Lasagna made the remarkable discovery, confirmed by Keats and Telford in 1956, that nalorphine, a narcotic antagonist, was an analgesic in man comparable in potency to morphine. This observation took an added significance when it was recalled that nalorphine was ineffective in the hot-plate and tail flick tests. Further clinical studies by Harris and Isbell at Lexington confirmed the belief that nalorphine did not and could not support morphine addiction. On the contrary it precipitated an abstinence syndrome in morphine-dependent addicts. Direct addiction studies in post-addicts revealed

that it did not induce a morphine-like dependence.

It occurred to us that the problem lay not on the chemical, but on the biological side. It should be noted that nalorphine met all of Eddy's structural requirements. The trivial chemical modification of exchanging an allyl for a methyl group exerted a profound effect on the pharmacological and clinical properties of the new drug. Perhaps our hallowed laboratory tests correlated better with addiction liability than clinical analgesic potency. When Eddy's review articles were examined from this vantage point, interesting correlations emerged. With the limits of accuracy of the experimental data the Spearman rank order coefficient correlating human addiction liability with mouse activity was just as high as that correlating this latter parameter with clinical potency. We culled seven well-studied analgesics from this group and did a rank order correlation on the following parameters: D'Amour-Smith potency, mouse tail-flick potency, clinical potency, and ability to support morphine addiction. The results are summarized in Chart I.

CHART I

FOUR PARAMETER RANK ORDER CORRELATIONS OF SEVEN ANALGESICS

	Mouse	Rat	Man	Addiction
Methadone	1	2	2	1
Isomethadone	4	3	4	4
Meperidine	5	5	5	5
Ketobemidone	2	1	1	2
Codeine	7	7	6	6
Morphine	3	4	3	3
d-Propoxyphene	6	6	7	7

Mouse vs. Rat	0.93	Rat vs. Man	0.93
Mouse vs. Man	0.93	Rat vs. Addiction	0.89
Mouse vs. Addiction	0.99		

It is clear what would happen to the correlation of laboratory activity with clinical analgesia if nalorphine were included. This analysis led us to conclude that the biological activity that was being measured had no positive relevance to the clinical goals that were being sought. Compounds that were active would almost certainly induce a morphine-like physical dependence in man, a situation we were trying to avoid. On the basis of these considerations we adopted a new tactical approach. On the biological side we

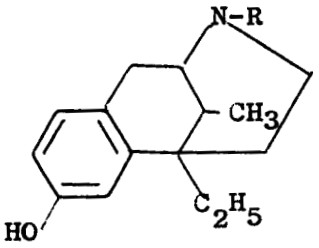
evaluated compounds as narcotic antagonists and rejected D'Amour-Smith positive compounds from further evaluation. On the chemical side we confined our synthetic efforts to those classes of compounds which fell within the scope of Eddy's postulates, except that we allowed the choice of the N-alkyl substituent to be guided by the activity as antagonists.

One of the first compounds studied was the N-allyl analog of meperidine which proved to be devoid of antagonist activity. Since levallorphan was a known narcotic antagonist of unknown, at least to us, clinical analgesic activity, we were interested in examining the corresponding cyclopropylmethyl analog as an antagonist. We were afforded this opportunity, but in a surprising way.

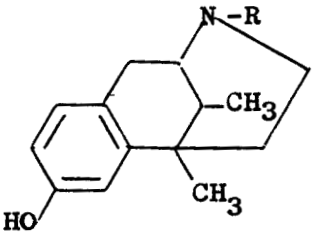
Several years ago I was requested to invite Marshall Gates to discuss at a business meeting of our Division editorial policy changes that the Journal of the American Chemical Society was instituting. He graciously consented to accept the invitation and at dinner that Sunday evening I casually inquired about his research program. To my astonishment he related how he thought that the narcotic antagonist approach afforded the best hope for finding a non-addicting strong analgesic and that he had a graduate student preparing N-cyclopropylnormorphine and N-cyclopropylmorphinan! As a result of this meeting, the S.W.R.I. group decided to forego work in the morphinan series and to concentrate its efforts instead in the benzomorphan series. We were pleased when Dr. Gates accepted our offer to test his series of antagonists and later one member of this series, cyclorphan, turned out to be an extremely active analgesic.

As is well known by now, we synthesized a large number of benzomorphans and evaluated several in the clinic. Only two have been subjected to the rigorous examination for physical dependence capacity in Lexington. Neither cyclazocine nor pentazocine substitute for morphine in dependent subjects nor do they induce a morphine-like primary dependence in post-addicts. There is no reason to believe that the other narcotic antagonists would behave differently. Some of the narcotic antagonists which have been studied over the past several years are shown in Chart II. The clinical analgesic activity is recorded in the last column of Table I.

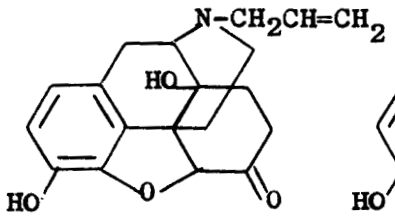
CHART II



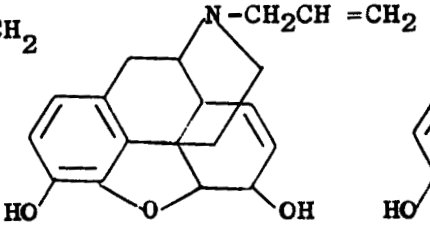
R = $CH_2CH=CH_2$ (Win 19,362)
R = $CH_2CH=C(CH_3)_2$ (Win 20,264)



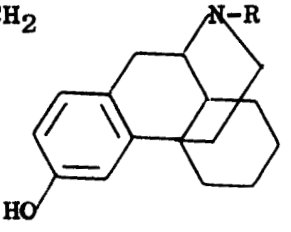
R = $CH_2CH=CH_2$ (SKF 10047)
R = $CH_2CH=C(CH_3)_2$ (Pentazocine)
R = $CH_2CH=CHCl$ (Win 29M)
R = CH_2CH——CH_2 (Cyclazocine)
(CH_2)
R = CH_2CH—CH_2 (Win 23,030)
(CH_2–CH_2)



Naloxone



Nalorphine



R = $CH_2CH=CH_2$ Levallorphan
R = CH_2CH——CH_2 Cyclorphan
(CH_2)
R = $CH_2CH_2CH_3$ NIH 6076

The search for a suitable biological assay in this field is continuing. A few years ago Sam Irwin, then at Schering, and Harold Blumberg of Endo Labs independently suggested that the phenylquinone writhing test devised sometime earlier was suitable for determining analgesic activity of narcotic antagonists. Harris and Pearl in our laboratory extended their observations and initially agreed that this technique was useful. Anne Pierson and I were skeptical. It had been felt for some time and recently confirmed by Chernov of Ciba that analgesic test procedures which are based on the reduction of writhing are rather non-specific. Our reservations were based on the knowledge that some of the compounds studied were powerful polysnyaptic blocking agents and it seemed likely that the reduction in writhing could be the result of the muscle relaxant activity of the drugs rather than their analgesic properties. We had in hand a number of narcotic antagonists of clinically determined analgesic potency and tried to settle this question by comparing their potency in the writhing test with their activity as muscle relaxants as measured by their ability to cause treated mice to fall off an inclined screen. The results are recorded in Table I.

The Spearman rank order coefficients for writhing vs. clinical activity and muscle relaxant activity vs. clinical activity were 0.67 and 0.7 respectively. The coefficient for relaxant vs. writhing activity was 0.8. These results confirmed our skepticism of the value of the writhing test in predicting analgesic activity of narcotic antagonists. Although a suitable small animal screening procedure for evaluating the analgesic potential of narcotic antagonists is not yet at hand, efforts in this direction should continue.

Great effort had been expended to find a non-addicting strong analgesic, but it was not until the limitations of the laboratory models were recognized that substantial progress was made. In a sense the status of research in schistosomiasis was similar. Drug evaluation in mice led to the discovery of Miracil D, the first orally effective, non-metallic schistosomicidal agent. Many congeners were synthesized; some of which were active in mice. However, tests in primates were erratic and clinical progress was disappointing. As we shall see, the difficulty lay not with the inadequacy of the laboratory models but with another aspect of biological activity.

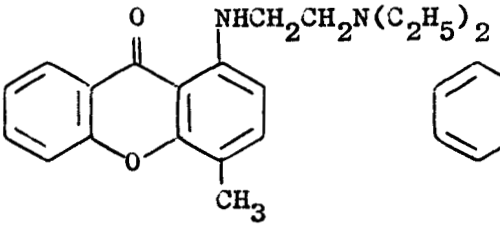
In a 1961 paper entitled "The Structure-Activity Relationship in Several Schistosomicidal Compounds" Gönnert summarized the progress to date. Some of the compounds he discussed are shown in Chart III, together with the indications of activity in certain species.

TABLE I

Results of Writhing Test, Inclined Screen Test and Clinical Potency of Narcotic Antagonists

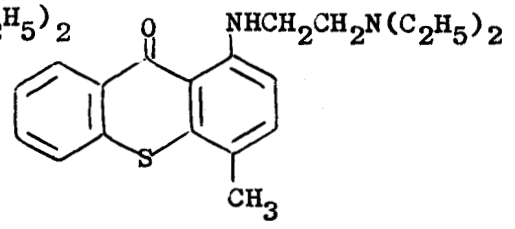
Compound	Writhing Test ED_{50} (95% confidence limits) mg/kg of case s.c.	Inclined Screen ED_{50} ± s.e. mg/kg of base s.c.	Clinical Equivalent 10 mg of Morphine Sulfate
Win 19,362	16.5 (12.0-22.8)	37.5 ± 1.8	5.0
SKF-10047	23.5 (16.0-34.8)	24 ± 1.5	15.0 +
Pentazocine	3.8 (2.1-6.8)	33 ± 3.7	30.0
Win 20,264	5.2 (4.2-6.4)	38 ± 3.0	40.0
Win 29M	25.3 (14.5-44.3)	23.5 ± 2.5	2.0 +
Cyclazocine	0.115 (0.07-0.2)	2.95 ± .17	0.25
Win 23,030	0.195 (0.130-0.293)	5.5 ± 0.3	1.0
Nalorphine	29.0 (20.1-41.8)	>200	Questionable
Naloxone	103 (83.1-27.7)	67% at 200	Inactive
Levallorphan	23.6 (13.9-40.1)	39.8 ± 4.2	12
Cyclorphan	0.055 (0.033-0.091)	15 ± 0.89	0.18
NIH 6076	35.0 (18.4-66.5)	54.2 ± 4.0	±

CHART III



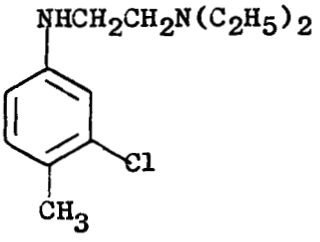
Miracil A

Mouse ±



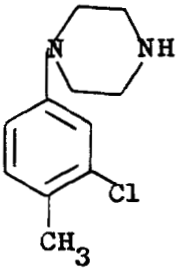
Miracil D

Mouse +
Monkey +++
Man ++



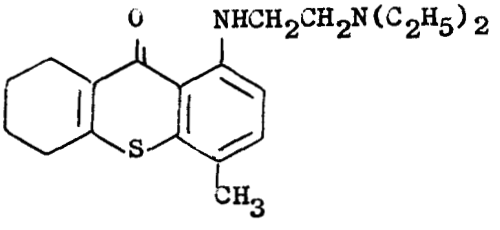
Mirasan

Mouse ++
Monkey o
Man o



S-688

Mouse ++
Monkey ?
Man o



PC-1941

"depends on species of infected host"

Gönnert concluded that:

1. "In several classes of compounds the schistosomicidal activity depends on a p-substitution of a methyl group and a basic side chain.

2. The efficacy may be increased by the addition of a substituent, particularly a chlorine atom in an alpha position to the methyl group.

3. The biological activity depends on the genus of the host even when the same strain of the parasite is used for infection."

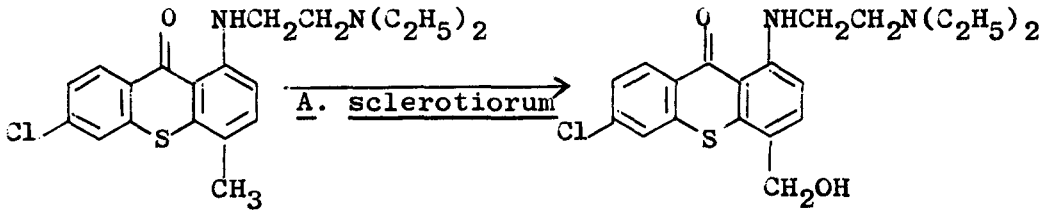
Gönnert warned that "the above conclusions as to the structure-activity relationships are based on the presumption that the compounds themselves and not one of their metabolites are responsible for the schistosomicidal activity". Strufe tried to identify the active metabolite of Miracil D but was not successful. Despite Strufe's failure, the notion persisted that the schistosomicidal activity of Miracil D was mediated through one of its metabolites. Why should the 6-chloro analog of Miracil D be about three times as active in the mouse, yet be totally inactive in the monkey? Why should Miracil D itself be much more active in the monkey, the species in which the metabolism is most extensive? These are just two questions which could be answered by the isolation of a biologically active metabolic product.

The isolation and identification in our laboratories of hycanthone as a microbiological transformation product of Miracil D and its subsequent evaluation as a schistosomicidal agent in hamsters furnished the first major clue that led to the solution of the problem. Study of the chemical properties of hycanthone showed that it was extremely sensitive to acid so that special precautions had to be taken when searching for its presence in the urine of monkeys medicated with Miracil D. Initial examination of such monkey urines revealed little if any of the putative metabolite. It seemed possible that the metabolite might be excreted as a conjugate of glucuronic acid. Pre-treatment of the appropriate urines and subsequent isolation revealed that next to Miracil D sulfoxide, hycanthone was the most abundant urinary metabolite. On the basis of these results we were encouraged to believe that hydroxylation of the methyl group was the key metabolic step. Are other congeners metabolized similarly? We turned our attention to 6-chloro Miracil D (V) because this drug is three times as active as

Miracil D in mice, yet it is ineffective in monkeys. If activation by hydroxylation is the key step, the hydroxy analog VI should be detectable as a mouse urinary metabolite, but not as a monkey conversion product. Accordingly, VI was prepared as shown in Chart IV.

CHART IV

Preparation and Activity of 6-Chlorohycanthone



V VI

6-Chloromiracil D
ED_{50}(mice) = 13.3 mg/kg

Miracil D
ED_{50}(mice) = 46.0 mg/kg

6-Chlorohycanthone
ED_{50}(hamsters) = 6.0 mg/kg

Hycanthone
ED_{50}(hamsters) = 0.93 mg/kg

The hydroxylated compound VI was easily detected in the urine of mice medicated with V, but despite a careful search in urine of monkeys similarly treated, we were unable to find VI even in trace amounts. The conversion product VI is much less active as a schistosomicidal agent than hycanthone in hamsters; the apparently greater activity of V in mice as compared with Miracil D is a result of more efficient enzymatic hydroxylation rather than of its intrinsic schistosomicidal activity.

Do all tri-cyclic congeners of Miracil D have to be activated by hydroxylation of the 4-methyl group? We answered this question in an indirect, but much less time-consuming manner. We prepared a series of hydroxylated analogs of hycanthone and compared their schistosomicidal activities in hamsters with that of their precursors, as shown in Table II.

Regardless of the type of substitution: replacement of the 6-H by 6-Cl (II-2), replacement of S by O (II-3), reduction of ring A (II-4), oxidation of S to SO (II-5) or change in the side-chain (II-6), the hydroxylated metabolite is the more active compound. We take this as strong, if not conclusive evidence, that in this series metabolic activation is a general requirement for schistosomicidal activity.

We were interested in determining whether this requirement held also for the structurally simpler compounds of Mirasan class. We focused our attention on Mirasan itself and the piperazine analog S-688 and prepared the corresponding hydroxymethyl derivatives both by purely chemical and microbiological methods. Before biological evaluation we predicted that Mirasan would be more effective in the mouse than in the hamster because of the much greater quantities of the corresponding hydroxylated metabolite in the urine of mice medicated with Mirasan than in the urine of hamsters treated similarly.

The results shown in Table III confirmed our prediction. The similarity in potency in mice between Mirasan (III-1) and its metabolites (III-2) suggest that (a) the enzymatic conversion is quite efficient; (b) that metabolic degradation and possible excretion of the latter is quite rapid or, (c) a combination of both effects is operative. The interspecies and intercompound differences in the S-688 pair (III-3 and III-4) are even more striking.

TABLE II

Schistosomicidal Activity in Hamsters of a Series of Congeners of Miracil D and Hycanthone

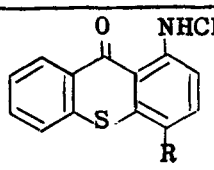
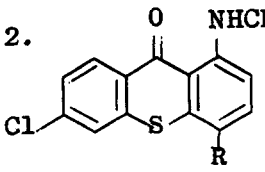
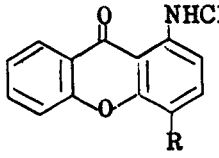
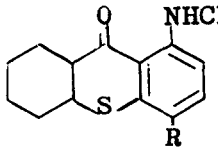
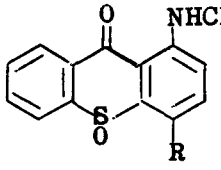
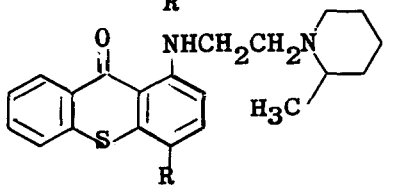
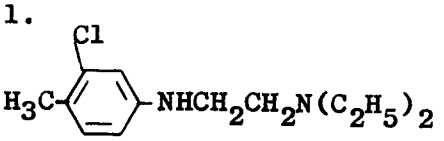
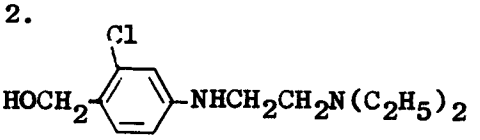
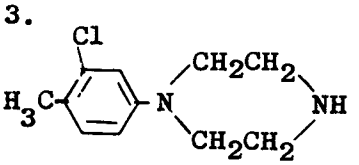
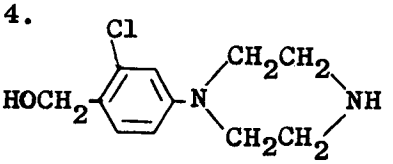
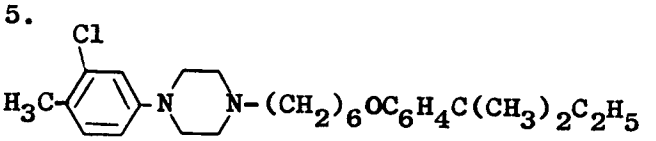
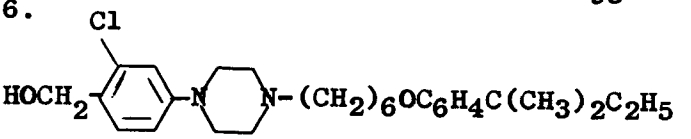
Compound	Schistosomicidal Activity ED_{50} mg/kg p.o. in Hamsters	
	$R=CH_2OH$	$R=CH_3$
1. 	0.93	8.0
2. 	6.0	> 25
3. 	10.6	22.5
4. 	4.8	> 50
5. 	3.1	> 50
6. 	1.8	7.8

TABLE III

Schistosomicidal Activity of Mirasan and Its Congeners in Mice and Hamsters

Compound	Schistosomicidal Activity ED_{50} mg/kg Mice	Hamsters
1. 	13.0	45
2. 	15.0	9.0
3. 	7.5	84
4. 	1.7	3.75
5. 	93	> 400
6. 	36	225

At the Annual Meeting of the American Society of Tropical Medicine and Hygiene in New York in 1964, a group from Abbott Laboratories discussed a series of S-688 analogs of which III-5 was the most interesting. Because of its structural and biological similarities to S-688 we thought that this compound owed its activity to metabolic hydroxylation also. Accordingly, when we returned to Rensselaer we prepared III-5 and III-6 and compared their schistosomicidal activities. We were unable to confirm the high potency of III-5 claimed by the original authors, but this may be due to differences in pharmaceutical availability of the two different samples -- a factor often neglected at the screening level. However, the predictably greater activity of III-6 in both species lent credence to our deductions.

This exercise in applied biochemistry has served to clarify some of the bewildering structure-activity relationships which have developed in this area of schistosomicidal research over the past two decades. The therapeutic significance of this work is another story which will be told in due time. Suffice it to say now that preliminary clinical trials with hycanthone are very encouraging. In Brazil Pellegrino has reported his early experience in treating about fifty patients suffering from S. mansoni infection. At doses of about 2.0 mg to 3.0 mg/kg given daily for five days over 80% cure rates were achieved and persisted for several months after treatment.

It is my view that the conventional wisdom concerning structure-activity relationships let us down in the two problems which I have just discussed. In the case of the analgesic antagonists, failure was due to a lack of relevance of the laboratory models to the desired clinical goals. In the case of the schistosomicidal agents, it was due to an underestimation of the importance of metabolic activation. About fifteen years ago Dr. Detlev Bronk asked the author of a biological paper what he meant by "activity". I hope that I have shown this evening that this question has as much contemporary significance as it did on the day it was asked.

Co-Authors on Medicinal Chemistry Papers and Allied Subjects

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

Chapter I. Antipsychotic and Anti-anxiety Agents

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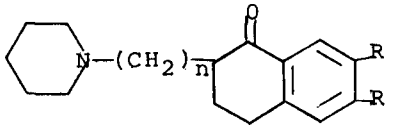
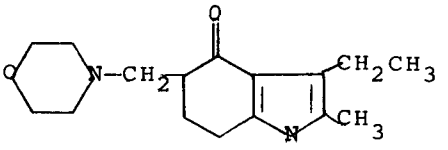
Newer Compounds in Clinical Trial

In 1959 the first of a series of papers appeared describing the potential antipsychotic activity of Mannich bases of tetralones.[1] 2-Piperidinomethyl-1-tetralone (NA-86, 1) was reported to be approximately one-third as potent as chlorpromazine in blocking conditioned avoidance and antagonizing stimulant effects of amphetamine in rodents.[2] Extending chain length decreased CNS depressant effects;[3] compound 2 was less active than 1 and more active than 3. Alkyl groups on the aromatic ring enhanced activity and N-702 (4) was most potent of a series of tetralones.[2]



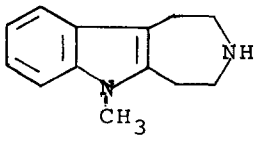
1, n = 1; R=H 3, n = 3; R=H
2, n = 2; R=H 4, n = 1; R=CH_3



5

The reported contribution of pyrrole nuclei to the sedative action of ketone derivatives[4] encouraged the synthesis of relatives of 4 in which the benzene ring was replaced by pyrrole. Molindone (EN-1733, 5), which is two to three times as potent as chlorpromazine in the rat, was among the most active and least toxic of the potential antipsychotic agents of the new series.[5,6] Aspects of its profile suggestive of antidepressant activity have been reported.[7,8] Two published studies describe the effective antipsychotic action of molindone in chronic schizophrenics. Unusual euphoria was noted in some patients.[9,10]

A new indole derivative, U-22394 (6), incorporates a part of the iboga alkaloid structure. U-22394 antagonizes the aggressiveness of fighting mice, is active in blocking conditioned avoidance, is hypothermic and anorexigenic and displays tryptamine-like activity in mice.[11] In chronic schizophrenics, U-22394 did not show antipsychotic activity.[12] Patients lost weight, suggesting possible anorexigenic effect.



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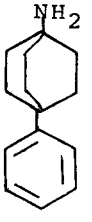

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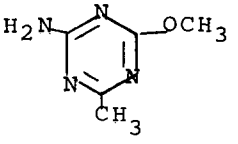
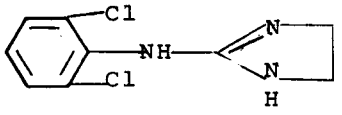


8

4-Phenylbicyclo(2,2,2)octan-1-amine(EXP 561,7), a potential antipsychotic of unusual structure, was studied in chronic withdrawn schizophrenics. A worsening of their condition was reported.[13]

Several case reports were cited to show that 2-amino-4-methyl-6-methoxy-1,3,5-triazine (8) is therapeutically effective in treating chronic schizophrenics.[14]

European studies with the effective antihypertensive drug Catapres (2-(2,6-dichlorophenylamine)-2-imidazoline, 9) uncovered useful anti-anxiety effects at 1 mg. per day and decreased agitation in psychotic patients at higher doses. Two attempts to confirm these findings in the United States were unsuccessful.[15,1] The drug was of no benefit to patients with agitation resulting from chronic psychotic illness.



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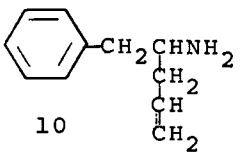

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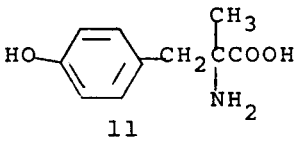
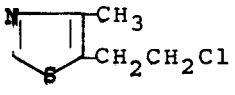


11

In preliminary studies with the potential analgetic compound aletamine (10), subjects reported "relaxed feeling blended with stimulation". To assess psychotropic potential, the drug was given to chronic schizophrenics. No psychotropic action was observed up to the time that limiting side effects caused termination of the study.[17]

α-Methyl-p-tyrosine (MK-781,11) inhibits tyrosine hydroxylase. Animal experiments showed that the compound reduced norepinephrine and dopamine levels in mouse and rat brain. Mild sedation or tranquilization was observed in monkeys and dogs. Two studies have been published assessing the potential value of α-methyl-p-tyrosine in schizophrenics. The compound was found to be without antipsychotic effect.[18,19]

The remarkably effective new drug for the treatment of delirium tremens, chlormethiazole (12)[20-22] provided control of extremely disturbed patients who proved unmanageable with conventional tranquilizers and sedatives.[22]



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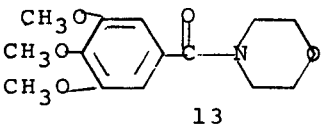

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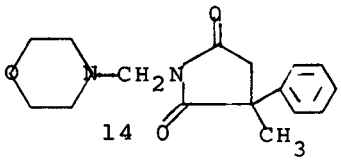


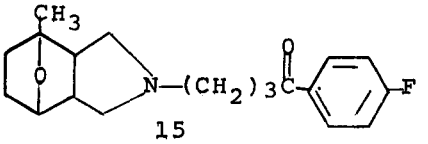
14

Trioxazine (13), previously described as a mild tranquilizer and effective daytime sedative, was evaluated for the treatment of chronic psychotic patients at doses as high as 2100 mg. per day. Useful psychotropic properties were not displayed.[23] Trioxazine appears to raise blood sugar levels.[23,24] Opinions differ as to whether it causes glycosuria.[23,25]

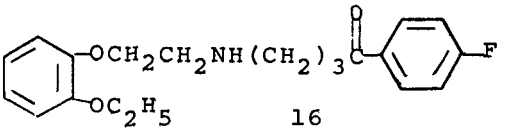
Morfolep (14), satisfactory in the management of petit mal,

was ineffective in treatment of psychomotor excitation.[26]

4,7-Bridged isoindolines were most potent of a new series of butyrophenones. 2-[3-(p-Fluorobenzoyl)propyl]-4-methyl-4,7-epoxyperhydroisoindoline (15) produced depression in rats for over six hours at a dose of less than 1 mg./kg. A reference to clinical efficacy of 15 was cited.[27]



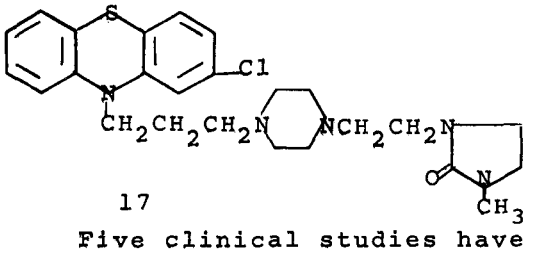
15



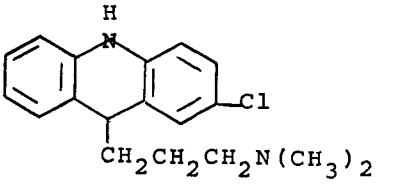
16

Another new butyrophenone, CI-601 (16), was found to possess neuroleptic properties, but at doses which produced marked extrapyramidal tremor.[28]

A new phenothiazine derivative, chlorimpiphenine (17), bears a third heterocyclic nucleus.[29] It is more potent and longer acting than perphenazine as an anti-emetic in dogs and as an inhibitor of conditioned avoidance response in rats. It is an effective and rapidly-acting antipsychotic agent in man.[30]



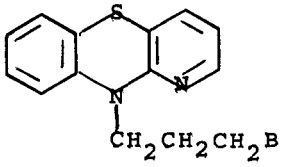
17



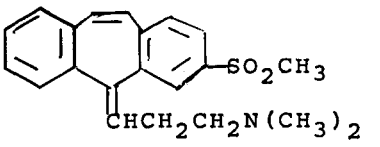
18

Five clinical studies have been published describing the action of the new acridane SKF 14,336 (18) in acute and chronic schizophrenia.[31,35] It has been described as notably effective with mild side effects;[32,35] slower in onset of action[34,35] and less active than chlorpromazine,[33] and limited by high incidence of side effects.[31,33] An attempt has been made to explain the different results obtained.[35]

Oxypendyl (19), prothipendyl (20) and MK-741 (21) were evaluated for antipsychotic properties. Each was said to have no likely place in the management of chronic schizophrenia.[36-38]



19, B=hydroxyethylpiperazino
20, B=dimethylamino

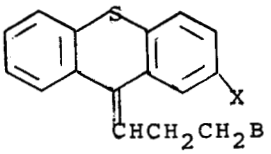


21

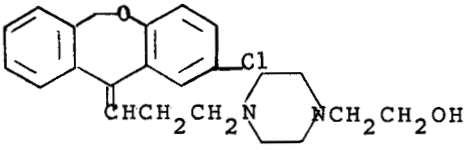
The properties of flupenthixol (22) were compared with those of clopenthixol (23) and chlorprothixene (24).[39] Flupenthixol was least sedative and most active in blocking conditioned reflexes in animals. It was least sedative and most effective

in patients with paranoid-hallucinatory schizophrenia. Chlorprothixene and clopenthixol were more effective in treating patients with psychomotor agitation.

Pinoxepin (25) was compared with thioridazine in a cooperative double-blind study. The drugs were equipotent. The former produced more extrapyramidal symptoms while the latter displayed more notable cardiovascular side effects.[40]

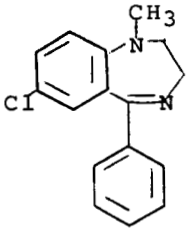


22, X=CF_3; B=hydroxyethylpiperazino
23, X=Cl; B=hydroxyethylpiperazino
24, X=Cl ; B=dimethylamino

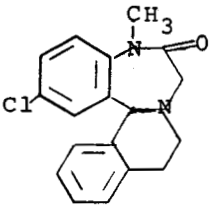


25

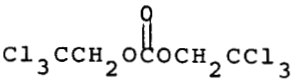
A new benzodiazepine, RO 5-4556 (26), proved effective in treatment of anxiety, tension and depression.[41,42] Side effects were minimal. Human mental and motor performance were not affected.[43]



26



27



28

Isoquinazepon (SAH-1123, 27), a benzodiazepine relative, was found to be without consistent anti-anxiety or antidepressant effect in man.[44] Collagen disease symptoms were reported following isoquinazepon administration to two patients.[45]

Chlorethate (28) was as selective as meprobamate in suppressing rage activity in mice without producing ataxia. In studies conducted in outpatient populations given medication for anxiety psychoneurosis, chlorethate was more effective than phenobarbital and equivalent in efficacy to meprobamate.[46]

Bufotenine has been detected in the urines of schizophrenics.[47,48] Methysergide (1-methyl-D-lysergic acid butanolamide), which attenuates or prevents the adverse effects of bufotenine, was evaluated as an antipsychotic drug. In one study it was reported ineffective,[49] in another it was said to have a deleterious effect.[50]

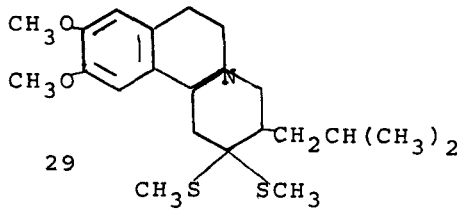
In 1966, diphosphopyridine nucleotide was publicized in the lay press as an effective drug for short term treatment of schizophrenia. This prompted further studies which found DPN to be of no therapeutic value to acute or chronic schizophrenics.[51,52]

The value of diphenylhydantoin in treating depression and anxiety was vividly described.[53] Although the drug appears to be

of little value to patients with psychoses severe enough to require hospitalization,[54,55] it may prove valuable to neurotic outpatients.[54,55] Carbamazepine, another anticonvulsant, may be even more effective.[55]

Newer Structures of Interest

Compound 29 is superior to tetrabenazine in mice.[57] Compound 30 produces locomotor depression in mice at 1 mg./kg.[58] Compound 31 blocks conditioned avoidance in rats.[59] Compound 32 is equal to chlorpromazine in blocking the aggressive behavior of fighting mice.[60] Compounds included in structure 33 showed tranquilizing and antidepressant actions without impairing alertness.[61] Compound 34, derived from 5-methoxytryptamine and pyridoxal, causes mice to be aggressive for weeks after single administration.[62]



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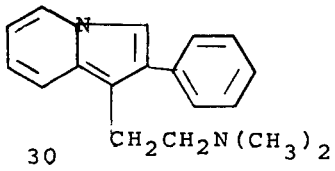

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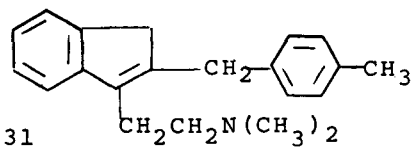

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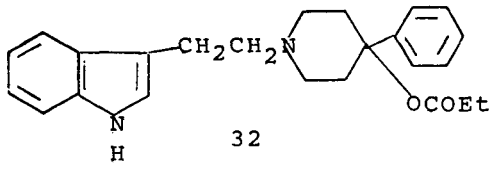

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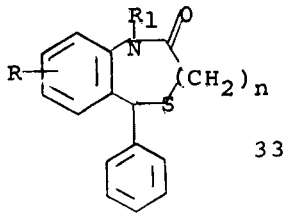

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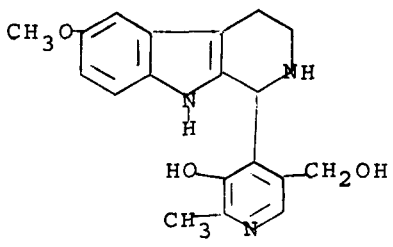

34

Notable Miscellany Relating to Clinical Drugs

The Food and Drug Administration approved New Drug Applications for use of butaperazine dimaleate, fluphenazine enanthate, haloperidol and thiothixene in treating schizophrenia.

The cholesterol lowering effects of trifluperidol, reported in 1966, were confirmed in further studies.[63,64] By contrast, haloperidol in large doses produced no lowering of serum cholesterol values or any deviation from the normal sterol patterns of patients in the study.

Tybamate in doses as high as 9 g. per day was abruptly withdrawn after 12 weeks of medication. Neither withdrawal symptoms nor evidence of drug dependence was observed. In this respect

tybamate differs from meprobamate.[65]

Oxazepam is produced in dogs as a metabolite of chlordiazepoxide.[66] Seventeen chlorpromazine metabolites were isolated from urine of psychotic patients in quantity sufficient for characterization.[68] Identification is simplified by the publication of infrared and ultraviolet absorption spectral data for chlorpromazine and 14 of its possible metabolites.[69] Butyrophenone metabolism, distribution and excretion studies were described.[70-74] p-Fluorobenzoylpropionic acid and p-fluorophenaceturic acid were identified as metabolites.[74]

Psychopharmacological Agents, Volume II was published. It includes important chapters on phenothiazines, butyrophenones and the biochemical basis of mental disease.[75] An entire issue of the International Journal of Neuropsychiatry was devoted to publication of a comprehensive symposium on haloperidol.[76] A compilation of data, primarily structural, on 690 psychotropic drugs and related compounds appeared.[77] Haloperidol[78] and thiothixene[79] have been reviewed with regard to clinical potential.

Central Nervous System Function of Neurohumoral Amines

In the past year, research emphasis has focused on the possible central transmitter functions of the catecholamines, norepinephrine and dopamine and the indolamine, serotonin. Three excellent reviews have considered this subject from the standpoints of transmitter criteria,[80] catecholamine activity[81] and amine influence on various affective states.[82] Pertinent data have been obtained from the following lines of investigation:

1) Identification of amine-containing structures along specific neuronal circuits in the central nervous system.[83,89]

2) Microelectrophoretic alteration of electrical activity in central neurons of the cat by neurohumoral amines.[84-88]

3) Uptake of radioactive norepinephrine in hypothalamic nerve endings and accumulation in dense-core vesicles.[90,92] Uptake of serotonin of the ventral part of the subarachnoid space.[91]

4) Mechanisms of uptake (transport across nerve cell membranes) and accumulation (binding at intraneuronal vesicles) of catecholamines and serotonin essentially similar by *in vitro*[93-95] and *in vivo*[96,97] techniques except for special pharmacologic conditions.[98,99]

5) Mystery of specific amine function unsolved by selective amine depletors.[100-103] Conflicting roles of serotonin in regulating body temperature.[103-105]

Antipsychotic Drug Mechanisms

Current theories of antipsychotic drug action are based on the observations that phenothiazines and related drugs increase the concentration of catecholamine metabolites in the brain (see Porter and Stone[106]). This effect has been attributed either to a direct activation of catecholamine neurons or to a compensatory feedback mechanism initiated by central catecholamine receptor blockade or by reduction of amine storage capacity.[107] Whatever the underlying events may be, antipsychotic drugs do modify central

catecholamine mechanisms.

For example, chlorpromazine enhanced central catecholamine biosynthesis *in vivo* by increasing the hydroxylation of tyrosine.[107-108] Chlorpromazine also accelerated cerebral catecholamine depletion in rats pretreated with a tyrosine hydroxylase inhibitor.[109-110] The latter experiments using histochemical fluorescence techniques[111] were taken to signify an increase in nerve impulse activity triggered by catecholamine receptor blockade and implemented by a compensatory feedback mechanism. The former could be treated in a similar hypothetical fashion, increased catecholamine biosynthesis representing a natural corollary to receptor blockade and enhanced catecholamine release. This cyclic process of blockade, synthesis and release would be interrupted when drug action diminished and adequate levels of intraneuronal catecholamines became re-established.[112-113] The finding that chlorpromazine blocked the central neuronal uptake of H^3-norepinephrine is germane to this thesis and consistent with the drug's action at peripheral sympathetic neurons.[114]

Certain pharmacologic actions of chlorpromazine have also been associated with central catecholamine function. Reduction of brain catecholamine levels in rats by inhibition of tyrosine hydroxylation enhanced the usual chlorpromazine-induced effects of hypothermia, potentiation of barbiturate hypnosis and blockade of a conditioned avoidance response.[115] That conditioned avoidance responses are dependent upon the activation of central catecholamine neurons has been demonstrated by histochemical and pharmacologic studies.[116,117]

Lithium salts, used successfully for some years in the management of manic states,[118] have recently been found to prevent relapses in manic-depressive psychoses and recurrent depressions.[119] Several lines of investigation have converged on the thesis that lithium may modify catecholamine function. *In vitro* data obtained from subcellular brain functions suggest that lithium effectively suppresses catecholamine activity by either increasing norepinephrine uptake[120] or decreasing norepinephrine stimulation of phosphatase activity.[121] In *in vivo* studies, lithium altered H_3-norepinephrine metabolism in rats by increasing the ratio of deaminated to O-methylated metabolites, suggesting enhanced intraneuronal inactivation of norepinephrine.[114,122] Finally Corrodi et al[123] found that the administration of lithium and a tyrosine hydroxylase inhibitor accelerated the depletion of norepinephrine from central neurons. These results indicate that lithium increases cerebral norepinephrine neuron activity either directly or by a receptor blockade-feedback mechanism.

The Biochemistry of Schizophrenia

Information continues to accumulate concerning the biochemical nature of schizophrenia despite the overwhelming and often forbidding complexities involved therein. Several comprehensive reviews[75,124-126] and one detailed research approach[127] have appeared in the past year.

Considerable effort has been directed toward the identifi-

cation of amine metabolites in the urine of schizophrenics. Most of these studies have their origin in the hypothesis advanced fifteen years ago by Osmond et al[128] in which pathologic transmethylation of catecholamines was considered to be a critical factor in the body's production of hallucinogenic, mescaline-like derivatives. This hypothesis was supported by the 1962 report of Friedhoff and van Winkle[129] which described the occurrence of 3,4-dimethoxyphenethylamine ("pink spot" on paper chromatograms) in schizophrenic urine. In the past year, a number of groups have attempted to confirm this finding, but only one investigation was substantiative;[130] two others supportive[131,132] and five others negative.[133,137] The possibility still exists that the "pink spot" is not a ring-methoxylated phenethylamine but rather a nonspecific metabolite derived from a dietary source, drug therapy or intestinal flora peculiar to life in an institution.

Another aspect of the transmethylation hypothesis involves the administration of methyl donors (L-methionine) or methyl acceptors (niacin, niacinamide) to schizophrenic patients in order to exacerbate or alleviate, respectively, the psychotic state. Although L-methionine appeared to intensify some schizophrenic symptoms (see Kety)[125], it did not increase the urinary levels of N- or O-methylated amines.[138] Moreover, the beneficial results reported by Hoffer et al[139] on the use of methyl acceptors in schizophrenia have not been confirmed by other investigators.[51,52]

A second general area of inquiry into the etiology of schizophrenia concerns the presence in the blood of an abnormal protein constituent. Heath and his colleagues have redirected their efforts on the protein factor, taraxein, along immunologic lines. In a series of three papers;[140-142] they reported that (1) schizophrenic serum contains an antibody that combines with neural cell nuclei, especially in the septal-basal caudate region of the brain, (2) the principle in schizophrenic serum which modifies the behavior of monkeys migrates with the gamma globulin fraction and (3) the similarity between the antibrain antibody produced in sheep and taraxein suggests that the latter is an antibody specific for brain. These interesting findings should provide the impetus for additional confirmatory and innovative investigations.

A plasma factor in schizophrenics, isolated by Frohman some years ago, was reported to raise the lactate-pyruvate ratio in a medium of incubating chicken erythrocytes. Re-evaluation of this fraction indicated that its action on carbohydrate metabolism probably reflects certain environmental influences rather than factors directly concerned with schizophrenia.[143] Other investigators could not alter the glucose metabolism of human leukocytes incubated with schizophrenic serum.

Although undetectable in earlier studies;[125] bufotenine was found in schizophrenic urine.[47,48,145] Agents which exacerbated symptoms (e.g. tranylcypromine) increased bufotenine excretion and those which caused clinical improvement (e.g., trifluperidol) decreased excretion.[47,145]

As the search for a biochemical basis of schizophrenia continues, Weil-Malherbe[124] emphasizes "the necessity of a blind design in this area of research even though the investigators be capable men of unquestioned integrity".

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Chapter 2. Antidepressants, Stimulants, Hallucinogens

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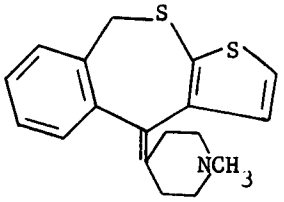
I. ANTIDEPRESSANTS

Chemistry - There were continued efforts to develop new thymoleptic agents of which imipramine is the prototype. A review of antidepressant drugs has appeared[1] and several important findings, dealing mainly with tricyclic agents, were presented at a Symposium held in Milan[2]. Hetero analogues of the tricyclic compounds frequently possess neuroleptic and antihistaminic rather than thymoleptic properties. The thiophene analogue (1) did exhibit a significant anti-reserpine effect which was weaker, however, than that found with the usual thymoleptics[3]. The dibenzoxepine (2) also possessed antireserpine and central cholinolytic actions[4]. Replacement of an aromatic ring in the dibenzocycloheptene series gave 3 which had antireserpine activity[5]. Mention was made of 10-keto-imipramine (4a) as a clinically-effective antidepressant, although a preliminary report did not indicate pronounced pharmacological properties (ref. 2, pp. 205, 397).

A number of basically-substituted phenylindenes and -indans having spatial orientations similar to those of the tricyclic drugs was prepared. Of these, the most interesting as a potential antidepressant (prevention but not reversal of reserpine-ptosis) was 5. It was more potent than imipramine or amitriptyline and had no central nor peripheral anticholinergic properties[6]. A review of biologically active indanes and indenes has appeared[7]. Certain 1-aminopiperidinols also inhibited reserpine-ptosis and were not MAO inhibitors. Compound 6 was the most promising[8]. A reduction product of strychnine (7) was reported to possess imipramine-like action in rats and there were no signs of convulsant activity[9]. Molindone (8), which is structurally unrelated to the known psychotropic drugs, possesses pharmacological properties found in both the neuroleptics and the thymoleptics. It could be of value where the combination of these drugs is indicated[10]. Other novel compounds reported at the Milan Symposium were the aryloxyacetamide, mefexamide (9) and an analogue of γ-aminobutyric acid (10). The former possesses several of the important properties of imipramine in animals as is relatively free from side effects while the latter, despite a favourable neuropharmacological profile, gave poor clinical results (ref. 2, pp. 208, 222, 360).

A number of compounds related to tranylcypromine (e.g. 11, trans) exerted antidepressant effects in animals qualitatively similar to those of the parent drug without, however, modifying brain or liver MAO activity[11]. A indanamine (12) was reported to be a very potent, long-acting and cumulative MAO inhibitor with a marked selectivity for brain MAO[12].

Pharmacology - An extensive pharmacological investigation of hepzidine (13) has revealed the expected autonomic effects together with several properties found in the thymoleptics (antagonism of reserpine, tetrabenazine and serotonin; central cholinolytic action)[13]. The benzylpiperidine 14 and the β-adrenergic blocking agent propanolol (Inderal^{R}) were suggested for



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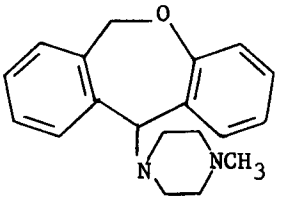

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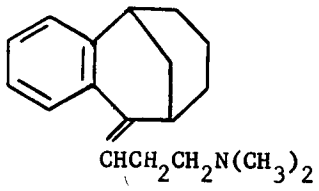

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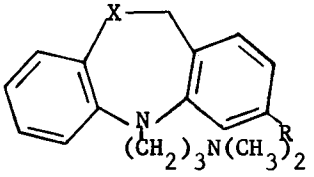
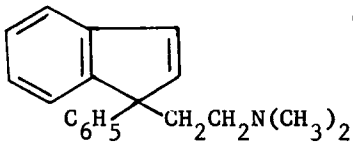


4a, X=C=O, R=H: G35,259
4b, X=CH_2, R=Cl: G34,586; Anafranil^R



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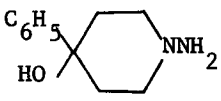

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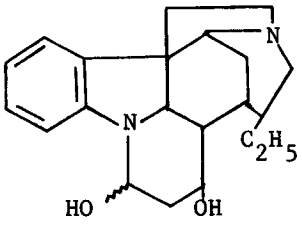

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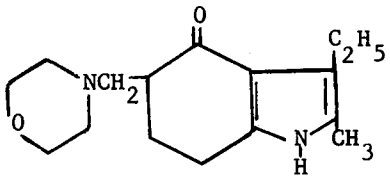
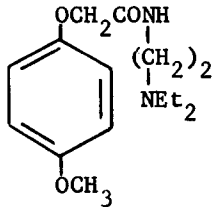
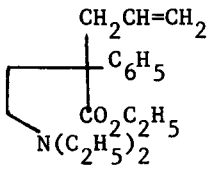


8 molindone (EN-1733A)



9 mefexamide (ANP-297)



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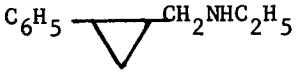
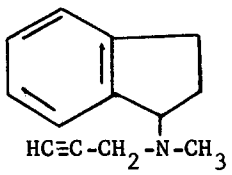


11

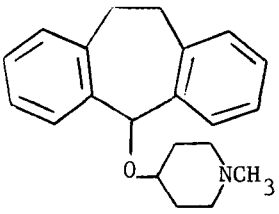


12 Su-11,739

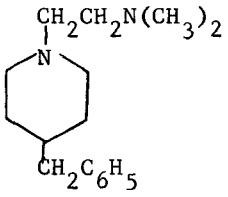
clinical trial as antidepressants on the basis of their dose-related reversal of Ditran-induced behaviour. [14,15] In a study of the cholinolytic properties of imipramine, amitriptyline and their N-desmethyl analogues, it was suggested that the drugs act by adrenergic rather than by cholinergic mechanisms. Their central effects resembled those of hyoscine rather than atropine [16]. A correlation was claimed, however, between antidepressant and central cholinolytic activity [17]. Imipramine was found to possess analgetic activity comparable to that of aspirin [8] and codeine [18] in the phenylquinone writhing test; related tricyclic compounds were even more

active. These drugs also enhanced the hyperthermic phase of reserpine [19]. Lapin [20] has reported a procedure for distinguishing between antidepressants and cholinolytics based on the qualitative differences in reserpine-antagonism and behavioural effects.

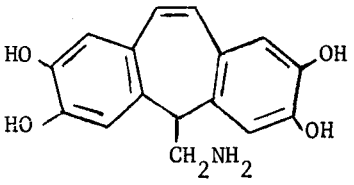
The dibenzocycloheptene derivative noradnamine (15), a proposed metabolite of norepinephrine (NE), has been suggested as a causative factor in depression. The hypothesis was supported by the finding that 15 was a potent inhibitor of COMT [21] and that when given intracerebrally to mice it caused marked akinesia and hypothermia; it failed, however, to potentiate barbiturate narcosis [22] and was shown not to be the mediator of NE-induced akinesia in mice [23]. Its sympathomimetic effects (i.v.) were exerted via adrenergic pathways, through a release of endogenous catecholamines [24].



13 hepzidine (BS-7051)



14 pimetine (IN-379)



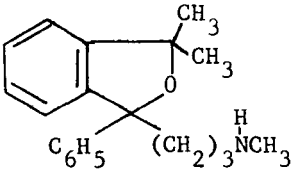
15 noradnamine

Biochemistry - There has been a vast amount of work reported on the biochemical processes involved in the affective disorders and space requirements preclude an adequate coverage. Several very useful reviews have appeared in 1967 [25 - 30, 30a]. Lithium salts may interfere with electrolyte balance and membrane transport [28](cf. ref. 2, p.80). Li^+ had no effect on brain NE content but when given together with a tyrosine hydroxylase inhibitor (methyl ester of dl α-methyltyrosine) it depleted NE; this effect was related to the Li^+ content in the brain [31]. Pretreatment of animals with Li^+ caused an increased uptake of NE by synaptosomes, possibly accounting for its anti-manic effect[32]. Li^+ also increases intraneuronal inactivation of NE (by deamination), resulting in lower levels of the amine available to central adrenergic receptors [33]. The phthalan Lu3-010 (16) was found to be the most specific inhibitor of the "adrenergic membrane pump" yet found. It was as active as protriptyline in inhibiting the uptake of ^3H-NE but was devoid of anticholinergic properties [34]. Results of a three-year study of catecholamine excretion patterns in depressive patients were reported [35]. In general, elevated urinary NE levels reflected acute psychological distress and the highest levels occurred during the most intense period of depression.

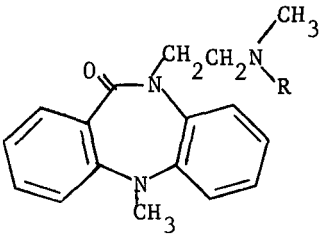
The role of dopamine was studied by several groups. l-DOPA temporarily reversed the reserpine-induced suppression of the conditioned avoidance response (CAR) and dopamine was the suggested mediator since (i) the time course of the reversal correlated with the rise in brain dopamine levels

and (ii) the reversal was blocked by prior treatment with a DOPA-decarboxylase inhibitor [36]. A similar conclusion was reached in a study of the selective reduction of dopamine levels in reserpine-treated mice [37]. The action of dopamine itself was studied using the selective inhibitors of NE synthesis, disulfiram (Antabuse^{R})[38] and diethyldithiocarbamate (DDC) [39].

About 70% of the radioactive dose of labelled imipramine was recovered in human urine; the results differ from those where unlabelled material was used [40]. Methods were reported for the estimation of plasma levels of desmethylimipramine (DMI) in patients receiving imipramine [41,42]. The rates of N-demethylation in vitro of a series of tricyclic psychotropic drugs were studied [43]. There was no evidence of demethylation of dibenzepine (17a) (see below) in rat brain [44] although 17b was found in the urine of humans receiving the tertiary amine [45]. In contrast to the results of previous workers, no DMI was found in rat brain after administration of imipramine [44]. DMI may potentiate the effects of amphetamine by a competitive inhibition of para-hydroxylation, thus raising its levels in brain and circulation [46]. It was recently shown that imipramine, unlike DMI, can selectively block the reserpine-resistant uptake-concentration mechanism in central serotonin neurons [47].



16 Lu 3-010



17a R=CH_3, dibenzepine (Noveril^{R})

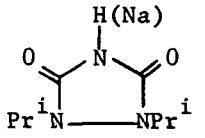
17b R=H

Clinical - Methods for evaluating psychotropic drugs were reviewed [48] together with a valuable cautionary note on the assessment of results [49]. A novel urazole derivative (18) showed antidepressant effects in a preliminary trial; it was effective in the DOPA potentiation test in animals and was not a MAO inhibitor [50]. In a controlled trial, 3-chlorimipramine (4b) was claimed to be as effective as imipramine with respect to speed of action, degree of effectiveness and nature of side-effects [51]. Pharmacologically, the drug was classed between imipramine and amitriptyline [52]. The indole derivative (19) was also reported to be an effective agent with lower toxicity and fewer side effects than imipramine [53,54]. Dibenzepine (17a) has gained clinical acceptance in Europe in cases where an antidepressant with mood-elevating properties is indicated [55-57]. Side-effects were generally not severe and the drug was reported to be particularly effective in combination with electroshock [58]. There has been increased interest in the use of lithium carbonate. It is effective in psychoses and manic-depressive disorders and has the important property of stabilizing mood and preventing periodically-recurring episodes of mania. It does not

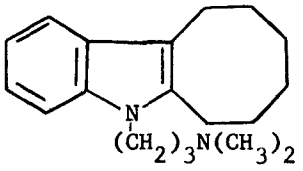
exhibit significant CNS depressant properties. Side-effects are infrequent but careful control of the dosage is required, especially when the drug is used prophylactically [59,60]. Instances of cutaneous reactions were reported recently [61].

The trend towards combining psychoactive drugs has been covered in a comprehensive review [62]. In addition to its combination with perphenazine (cf. 1966 Annual Reports, p. 16), amitriptyline was reported to be of value when given with chlordiazepoxide [63] or with protriptyline (20) [64] while 20, when given with meprobamate, was effective in cases of neurotic depression in a well-designed trial [65]. The combination of tricyclic thymoleptics with MAO inhibitors, a regimen contraindicated by some, was reported to result in a good improvement rate without causing serious side-effects (ref. 2, p. 336; ref. 62, p. 7). Several imipramine-resistant patients were benefited when reserpine was given concomitantly; marked vasodilatation and increased intestinal peristalsis was observed [66].

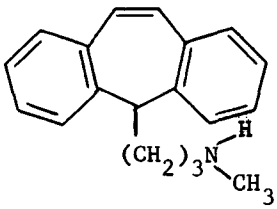
The possible role of steroid hormones in depression has received attention and the induction of mood and behavioural changes by the oral contraceptives was discussed [67]. The conflicting reports on a possible correlation between elevated 17-hydroxycorticosteroid levels and depressive states were reviewed [28,68,69a]. Recent papers have not resolved the problem [69b,70-72]. An interesting rationale for future psychoendocrine research was presented which emphasized the need to study the interrelationships of the steroid hormones, biogenic amines and electrolyte balance [68]. Mention was made of a pilot study where patients previously resistant to antidepressants were benefited by prednisone [73]. The opportunity to develop novel psychoactive steroids remains a challenge to medicinal chemists.



18 (A-10749)



19 iprindole
Pramindole^R

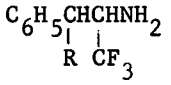


20 protriptyline
Vivactil^R

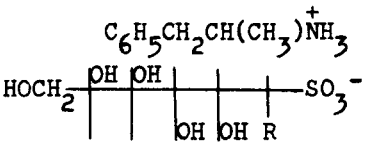
II. CENTRAL STIMULANTS

Chemistry - In contrast to the efforts in the field of antidepressants, relatively little work on central stimulants was reported. The structure-activity relationship of amphetamine derivatives was studied by a molecular orbital method [74]. The greatest central stimulant effect was associated with those compounds having the lowest negative charge on the ring carbon atom ortho to the side-chain and with the lowest dipole moment arising from

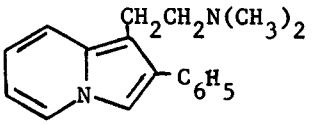
the conjugated portion of the molecule. The results suggested that hydrogen-bonding to the β-centre of the receptor. The α-trifluormethyl analogues of amphetamine and norephedrine (21) were essentially devoid of stimulant and pressor activities [75]. Mannose amphetamine sulfonate (22a) was somewhat more active and longer-acting as a locomotor stimulant than d-amphetamine sulfate; the related mannamine (22b) was inactive [76]. A number of 2-phenylindolizines caused CNS stimulation or depression in mice, depending on the dose; compound 23 produced stimulation in cats [77]. A new synthesis of diphenylisopropylamines (24), claimed to have long-acting stimulant properties, was described [78]. The method involved Friedel-Crafts reaction of ephedrine with aromatic hydrocarbons or heterocyclic compounds.



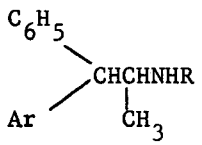
21 R=H,OH



22a, R=OH
22b, R=C_6H_5NH



23



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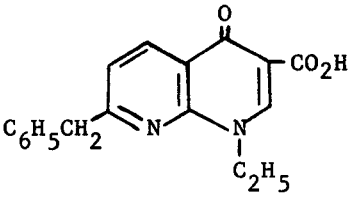


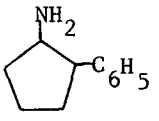
25

Biology - The naphthyridine derivative (25) was more potent than amphetamine as a locomotor stimulant. The effect was antagonized by reserpine and phenoxybenzamine but not by α-methyltyrosine or the selective serotonin (5-HT) depletor, p-chlorophenylalanine. It was suggested that the compound acts altering NE uptake or by releasing NE from the storage pool [79]. A series of tryptamines exhibited stimulant activity (body tremors in mice) which was related to their ability to inhibit the uptake of 5-HT; the stimulant effects correlated in part with their MAO inhibitory properties [81]. β-carbolines related to harmine, while more potent MAO inhibitors [80], did not interfere with 5-HT uptake and were not stimulants [81]. A new and relatively simple animal behavioural test which can distinguish between central excitants, antidepressants and depressants was described [82]. The method is based on the latency period to enter a hole of predetermined diameter.

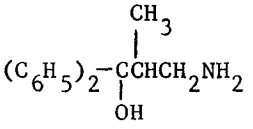
The question of the mechanism of action of amphetamine was discussed in previous Annual Reports (1965, p.23; 1966, p. 18). Further evidence was presented supporting the view that the central stimulatory effect is dependent on the availability of newly-synthesised NE [83,84]. Others suggested a direct action on central NE receptors [85] or a modulation of the degree of inhibition of MAO [86]. It was also proposed that the locomotor effect is associated with NE while dopamine may be involved in the stereotyped be-

havioural patterns [39,87,88].

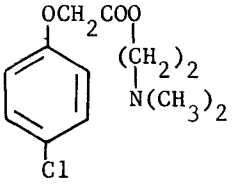
Clinical - Some improvement was observed in retarded children treated with cypenamine (26); the drug caused no untoward side-effects.[89] A controlled trial of U-23,807A (27) in patients with neurotic-, involutional-, or reactive depression indicated that the drug-treated cases fared no better, and possibly worse, than those receiving placebo [90]. The basic ester meclofenoxate (28), reported to possess central stimulant properties in animals [91], had no beneficial effect in geriatric patients with disorders of memory or mental concentration [92]. Further reports on magnesium pemoline (29)(cf.1966 Annual Reports, p. 18) indicated that the drug is of little clinical value in increasing memory performance or learning[93,94], although others have found it effective when given over a period of time [95]. It was suggested that its action on the CAR in rats [96] is due to an effect on performance systems rather than on learning and memory processes [97]. The ability to influence brain RNA [98] or protein synthesis was disputed [99]. A related compound, thozalinone (30), was not superior to placebo in patients with reactive or endogenous depression [100]. The use and abuse of central stimulants and the problems of dependence have been reviewed [101,102].



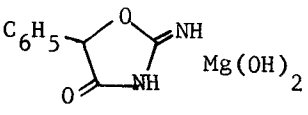
26 cypenamine



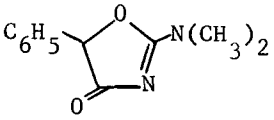
27 U-23807A



28 meclofenoxate (centrophenoxine) Lucidril®



29 Mg pemoline Cylert®



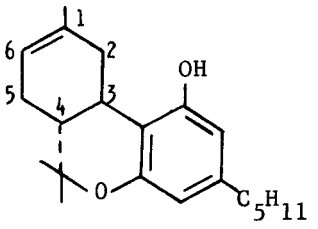
30 thozalinone

III. HALLUCINOGENS

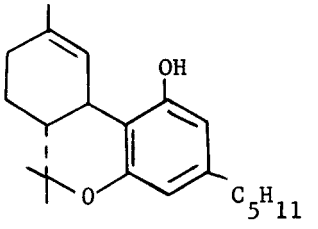
Chemistry - Considerable interest in the field of hallucinogenic agents was shown and notable developments included studies of novel phenethylamines having striking activity and important investigations of marihuana components. The latter topic was covered in an excellent review [103] which included several citations of the 1967 literature. An extensive monograph on the hallucinogens was published [104] as well as shorter surveys of the field in general [105,106] and the psychotomimetic glycollates in particular [107]. Interesting accounts of the search for psychoactive drugs from native plants have been described [108].

Significant advances in the chemistry of the cannabinoids have been made in recent years and several syntheses were reported in 1967. Of particular interest was the elegant method of Mechoulam et al.[109] involving condensation of (−)-verbenol with olivetol to give the psychoactive (−)-$\Delta^{1(6)}$-3,4-trans-tetrahydrocannabinol (THC)(31) which in turn was converted to (−)-Δ^{1}THC(32); the overall yield of 32 was 21%. Using a similar approach,

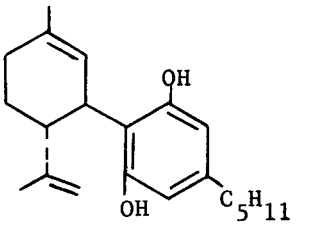
a Swiss group developed a one-step preparation of (−)31 [110] and quantitatively isomerised it to (−)32 [111]. These workers also developed a direct preparation of (−)-cannabidiol (33) [112] which could readily be converted to (−)31 or (−)32 (cf. ref. 109). A two-step synthesis of (−)31 involving resolution of an intermediate (34) [113] and a somewhat lengthy preparation of dl-31 and 32 [114] were reported. The unnatural (+)-enantiomorph of 31 was prepared [109,113] and found to be devoid of ataxic activity [109]. No pyrolytic cyclisation of cannabidiol to THC or isomerisation of 32 to 31 during the smoking of hemp was observed [115]. It was suggested that the Δ^1-THC acid (35), found in Mexican hemp, is decarboxylated on storage to form Δ^1-THC; the acid had no effect in the mouse catalepsy test [116]. An analogue (36) of Δ^3-THC was biologically inactive [117].



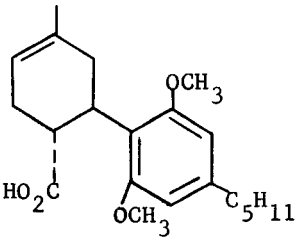
31 $\Delta^{1(6)}$-THC



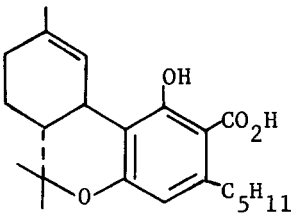
32 Δ^1-THC



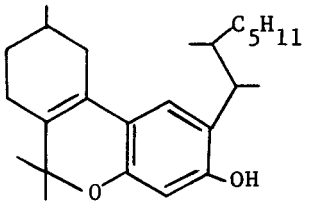
33 cannabidiol



34

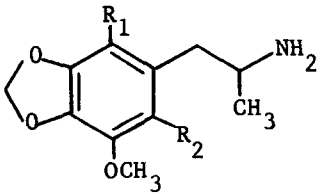


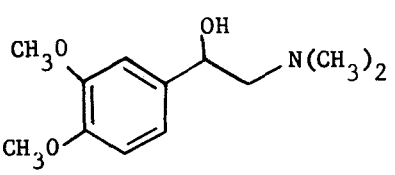
35 Δ^1-THC acid

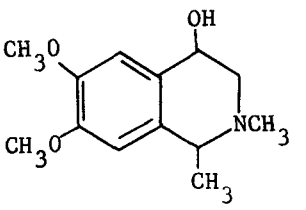


36

Using animal tests which are predictive of psychotomimetic activity in man, Smythies and co-workers [118] investigated the nineteen possible methoxylated phenethylamines. Three compounds were active in disrupting behaviour: 2,3,4,5,6>2,3,4,5>3,4,5(mescaline), the others being inactive. In the amphetamine series the tri-(2,4,5>3,4,5>2,4,6) and 3,4-dimethoxylated derivatives were active while 4-methoxyamphetamine was the most potent hallucinogen tested [119]. This finding has important implications with respect to the 4-methoxylation of the catecholamines, tyramine or amphetamine itself. Many of the known psychotropic amphetamines possess ring-substitution patterns identical to those of natural essential oils. Two novel methylenedioxy derivatives (37,38), prepared from apiole and dillapiole respectively, exhibited effects in humans which were described as intoxicating rather than psychotomimetic [120]. Two new hallucinogenic cactus alkaloids (39, 40) have been identified [121].







37 R=OCH_3, R_2=H (DMMDA) 39 macromerine 40 gigantine
38 R=H, R_2=OCH_3 (DMMDA-2)

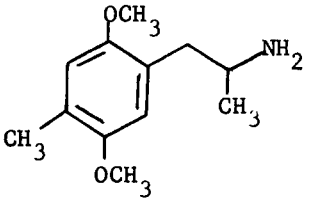
Biology - The pharmacology of the hallucinogens was reviewed briefly[122]. In a series of substituted tryptamines a statistically significant correlation was found between the respective potencies in evoking hyperthermia and open-field behavioural effects. It was suggested that the compounds act on the same CNS receptors as LSD [123]. Based on their effects on the CAR in rats, it was suggested that 5-methoxy-N,N-dimethyltryptamine should be a potent hallucinogen in man while the N-monomethyl analogue should be inactive [124] (cf.ref. 104, p. 458). Other animal tests which can predict hallucinogenic action in humans include the capacity to produce head-twitching in mice [125], studies of characteristic EEG patterns [126,127] and, in the psychotomimetic anticholinergics, interspecies correlations of effects [128]. Votava et al.[129] reported that LSD elevated serotonin and decreased NE and dopamine levels in the brain. In rats pretreated with reserpine, large doses of LSD evoked unexpected behavioural changes.

Amongst the proposed endogenous hallucinogens, the amounts of 3-4-dimethoxyphenethylamine (DMPEA) in rat brain correlated directly with performance in the rope climbing test [130]. It had effects similar to those of mescaline on classically conditioned avoidance behaviour in rats [131]. Its role in schizophrenia, however, remains controversial. DMPEA was only a minor constituent of the "pink spot" from the urine of schizophrenic patients and at least seven other compounds were present [132]. Another investigation showed that the pink spot material from some schizophrenics and most Parkinsonian patients was not DMPEA, but p-tyramine [133]. The lower levels of free dopamine in the urine of schizophrenics may reflect a faulty metabolism of this amine to give abnormally high levels of NE and epinephrine [134]. The presence of the known psychotogen, bufotenin, in the urine of schizophrenics has been confirmed using sensitive analytical methods [135]. Further work indicated a possible correlation between free bufotenin and exacerbation of psychotic behaviour [136].

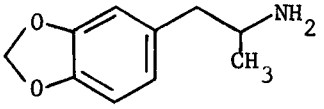
Clinical - A recent addition to the hippie armamentarium, STP, contains 2,5-dimethoxy-4-methylamphetamine (DOM,41) as the active component. Doses of 3 mg. or more in man produced hallucinatory effects lasting up to eight hours but,contrary to other reports, these effects were not intensified by chlorpromazine. The hallucinogenic potency is about 100 x mescaline and 1/30 x LSD [137]. A related compound, MDA(42), caused heightened affect, emotional empathy and access to feelings which suggested its use as an adjunct to psychotherapy. It did not produce the perceptual alterations or depersonalization found with other hallucinogens[138] The psychotomimetic

effects in man of pure (−)-Δ^1-tetrahydrocannabinol (32) were reported to be similar to those of LSD or cocaine: euphoria and alterations of visual, auditory and temporal perceptions. On smoking it was 2.6 to 3.0 times as potent as orally-ingested material [139]. Adverse reactions to marihuana [140] and the problem of dependence [141,142] have been reviewed.

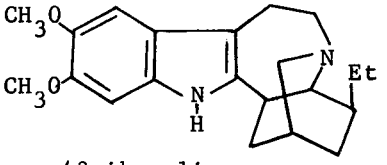
Amongst the indoles, 6-fluoro-N,N-diethyltryptamine (6-FDET) produced some of the physical and mood changes of the hallucinogenic N,N-diethyl- and N,N-dipropyltryptamines. It did not, however, induce the perceptual and thinking changes of these agents and was suggested as an "active placebo" in evaluating the therapeutic ability of hallucinogenic drugs [143]. Ibogaline (43) produced a state of intoxication but not the profound changes found in model psychoses [144]. Reports have appeared concerning the short- and long-term dangers of LSD [145,146]. The findings that it is linked to chromosomal and teratologic damage merit serious attention [147-149].



41 DOM



42 MDA



43 ibogaline

SUMMARY

Efforts to develop new antidepressants continue on three major fronts; (i) new tricyclic compounds have been studied whose properties range from thymoleptic to neuroleptic and whose subtle differences can be used to treat specific types of depression, (ii) a number of novel and unrelated structures are under investigation suggesting that drugs with properties quite different from those in current use may be available shortly and (iii) an increasing body of knowledge is being assembled concerning the biochemical processes involved in aberrant mental states. The use of lithium salts in manic-depressive disorders has demonstrated the need to study these processes in an integrated manner and a better understanding is required of the interactions between endogenous steroid hormones, electrolytes and the biogenic amines.

The interest shown in certain mild central stimulants capable of increasing memory and learning performance indicates the need for drugs in this field. While clinically useful agents have not yet been found, the techniques developed thus far may be of considerable value for future research.

In the field of the hallucinogens, several structurally simple compounds have been developed which are potentially useful as adjuncts to psychotherapy. Synthetic work on the components of marihuana has furnished pure compounds for more detailed evaluation and molecular modification of the cannabinoids may lead to new types of psychotropic agents. The illicit use of hallucinogens with the attendant psychopathological consequences continues to be a pressing problem.

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

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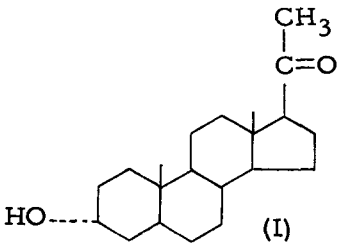
Introduction - Following the format of previous editions of this chapter the discussion of compounds is divided on the basis of established or projected clinical use rather than on the basis of pharmacological properties or chemical structure. Because of the relative ease of observing CNS depressant properties of new chemicals in small animals the literature is replete with reports of such agents. Only those compounds with more than preliminary pharmacological screening data were selected for inclusion in this review.

Hansch[1, 2] and coworkers, in their continuing attempts to place the discussion of structure-activity relationships of biologically active compounds on a mathematic basis, have presented evidence that the hypnotic activity of groups of barbiturates depends almost entirely on their relative lipophilic character as defined by their octanol-water partition coefficients. Although hypnotic activity was the model, the principle should apply to other actions of drugs in the central nervous system.

Laycock and Shulman[3], in a quantitative investigation of the pharmacological interaction of pairs of closely related β,β-dialkylglutarimide homologs with each other or with a series of structurally related or unrelated depressant drugs, provides good evidence for the idea that stimulant, depressant or dual stimulant-depressant actions produced by these drugs all arise at common sites in the CNS of the mouse.

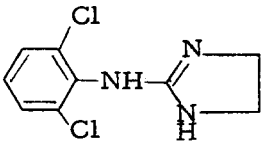
Sedatives and Hypnotics - Since the report of Selye[4] in 1942 concerning the hypnotic effect of certain steroids, a large number of these compounds - both hormonal and nonhormonal - have been shown to exhibit such effects[5, 6]. In a structure-activity study of the hypnotic properties of 62 steroids belonging to different chemical classes, Gyermek and coworkers[7] report that a few members of the 5α- and 5β-pregnane and 19-norpregnane class were outstanding as hypnotic agents in the mouse and rat. The water-soluble succinates of the potent pregnane derivatives were uniformly less effective and slower acting than the free alcohol and ketone forms. Pregn-4-enes, pregn-5-enes and the few androstane- and estrane-type steroids studied exhibited negligible hypnotic activity.



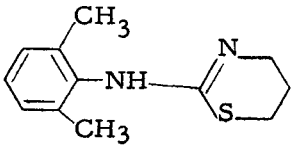
(I)

A detailed neuropharmacological study[8] of one of the compounds, pregnanolone (I), a metabolite of progesterone, was reported. It was found to be a highly potent, short-acting hypnotic agent when administered intravenously.

A clinical study[9] in chronic schizophrenics employing catapres (II), under investigation as a highly active antihypertensive agent, demonstrated a marked sedative effect of the compound; however, no improvement was seen in the disturbed behavior or agitation of the patients that resulted from their psychotic illness.



(II)

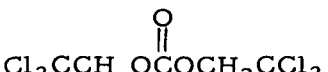


(III)

The related experimental hypotensive, Bayer 1470 (III) was reported to produce analgesia combined with a state of sleep in animals and man[10].

Chlorethate (IV)[11], a derivative of trichloroethanol, was compared clinically with phenobarbital and meprobamate[12]. It was significantly better than phenobarbital in out-patients with anxiety but there was no clinically significant difference when compared with meprobamate.

The o-, m-, and p-chloro substituted compounds related to methaqualone (V), were studied[13] from the viewpoint of hypnotic and anticonvulsant activity. The ortho compound, mecloqualone (VI), was the most active.



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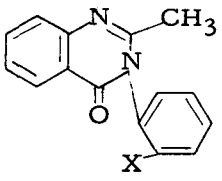


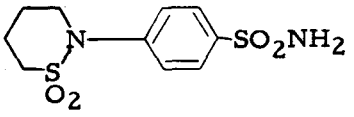
	X
(V)	CH_3
(VI)	Cl

Introduction of a second substituent on the phenyl nucleus reduces activity or increases toxicity.

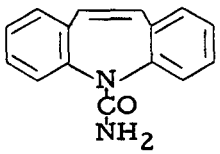
Anticonvulsants - Many laboratories routinely screen new compounds for their ability to modify chemically and electrically induced convulsions. Annually a large number of compounds are reported to have anticonvulsant activity, and 1967 was no exception. Cited here are only selected examples - generally those which have shown promise in man.

A conference of opinion leaders and active workers in the field was held in August 1967 by the U. S. Food and Drug Administration for the purpose of discussing the pharmacological, toxicological and clinical evaluation of anticonvulsant drugs[14]. This initial conference is to be followed by others dealing with more specific aspects of the problem. It was generally agreed that despite the variety of useful drugs in this field, new types of agents as well as superior ones to replace those now available are urgently needed. Summaries of the meeting have been issued by the FDA.

In separate reports Livingston and coworkers have reported clinical studies with sulthiame(VII)[15] and carbamazepine (VIII)[16]. Both drugs are commercially available in Europe and currently under investigation in several centers in the U. S. Sulthiame is reported to possess particularly significant anticonvulsant properties against major motor and psychomotor seizures, while carbamazepine was most useful in the latter form of epilepsy.

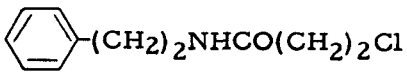


(VII)

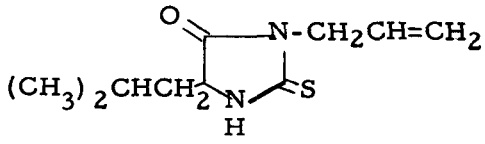


(VIII)

Phenacone (IX) was reported[17] as being effective in preventing convulsions caused by strong acoustic stimulation, electric shock and pentylenetetrazole in rats. In man it was effective in preventing psychomotor attacks but was less active against grand mal seizures.

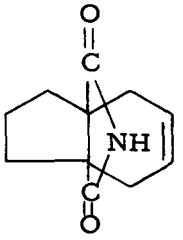


(IX)



(X)

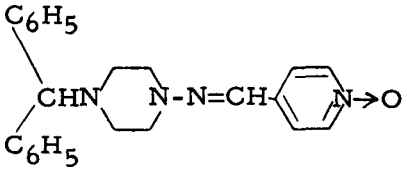
Comparison of albutoin (X)[18], a new thiohydantoin derivative, with diphenylhydantoin in a double-blind controlled study showed that the anticonvulsant potency was equal for the two and that albutoin was particularly effective against grand mal seizures and the incidence of side effects was slight and infrequent as compared with those from diphenylhydantoin. The anticonvulsant properties of this compound in laboratory animals were described earlier by Gesler and associates[19].



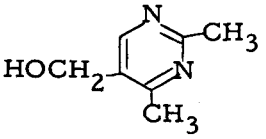
(XI)

Among the many new anticonvulsant succinimides reported, compound XI and related analogs described by Wagner and Rudzik[20] are structurally interesting.

Craig[21] reported a series of benzhydryl-piperidine and -piperazine compounds. The most active, SC-14740 (XII), may be equal or superior to diphenylhydantoin in the mouse.



(XII)



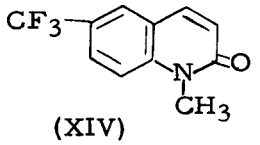
(XIII)

The convulsant properties of DHMP (XIII), previously reported by Schwan et al[22] was evaluated by Banziger and Hane[23] and was shown to be a useful tool in anticonvulsant screening to substantiate anti-electroshock activity.

Central Muscle Relaxants - There continues to be considerable controversy as to whether this group of medicinal agents is effective clinically by the oral route. Their effectiveness in animal experiments and when administered parenterally to humans is well established[24]. The need for a centrally acting skeletal muscle relaxant which is orally effective at a reasonable dose continues to exist.

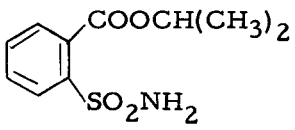
During the past year there were many compounds synthesized as potential central muscle relaxants[25, 26, 27, 28] but no encouraging evidence of any being promising as a clinically useful agent was presented. However, several compounds are worthy of mention as possible candidates for further study or models for additional synthetic work.

The pharmacology of SKF 13059 (XIV), reported by Mirsky and coworkers[29], demonstrates that this agent can be characterized as a centrally acting muscle relaxant with a component of "analgetic" activity. This quinolone derivative represents a new structure type in the muscle relaxant activity class.



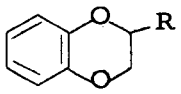
(XIV)

SKF 7711(XV) is a relatively weak and short acting drug[30]. In the dog and monkey it is somewhat less effective than meprobamate, zoxazolamine or chlormezanone, and much less so than chlordiazepoxide or diazepam. Unlike most muscle relaxants of this type, it is a selective antagonist of strychnine-induced convulsions; however, it shares this property with metaxalone and to a lesser extent with mephenesin.



(XV)

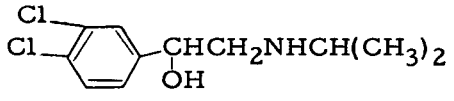
Benzodioxane derivatives continue to be studied for this activity. Two compounds, SAS-506 (XVI)[31] and MPB (XVII)[32], were reported to be worthy of continued study.



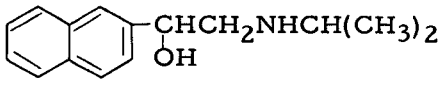
(XVI) $-\underset{\underset{OH}{|}}{CH}-CH_2O\overset{\overset{O}{||}}{C}-NHCH(CH_3)_2$ (R underlined)

(XVII) $-CH_2NHCH_2CH_2CH_2OCH_3 \cdot HCl$

The β-adrenergic blocking agents, DCI (XVIII), nethalide (XIX) and propranolol (XX) possess central muscle relaxant activity on the linguomandibular reflex in the cat according to Sinha et al[33]. The authors conclude that this appears to be related to their local anesthetic activity.



(XVIII)



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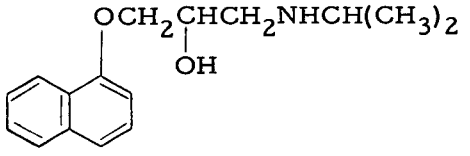
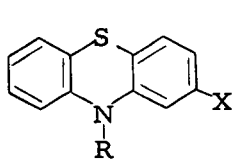


(XX)

More specific depression of γ-motoneurons seems to offer additional advantages in the treatment of skeleto-muscle disorders due to brain damage. For the most part, this has been a disappointing area for treatment with muscle relaxants that depress spinal interneurons. The finding that chlorpromazine (XXI) reduced the discharge of γ-motoneurons (Dasgupta and Werner, 1955)[34] led Keary and Maxwell[35] during the past year to study other phenothiazines in an effort to find drugs with specific action on this system without having significant sedative properties. Acepromazine (XXII) was shown to be 17 times more potent than chlorpromazine but it was possible to separate skeletal muscle relaxant activity from sedative activity only with chlorproethazine (XXIII), dimethothiazine (XXIV) and metopimazine (XXV). The latter two had one-fourth the potency of chlorpromazine on γ-motoneurons but were found to be considerably less sedative.

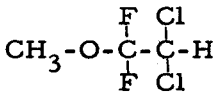


	X	R
(XXI)	-Cl	$-(CH_2)_3N(CH_3)_2$
(XXII)	$-COCH_3$	$-(CH_2)_3N(CH_3)_2$
(XXIII)	-Cl	$-(CH_2)_3N(C_2H_5)_2$
(XXIV)	$-SO_2N(CH_3)_2$	$-CH_2CHN(CH_3)_2$
(XXV)	$-SO_2CH_3$	$-(CH_2)_3N$ (CH$_3$ on ring) $-CONH_2$

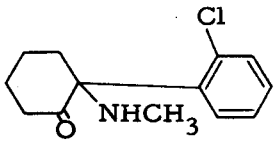
General Anesthetics - Although there were many reports concerning the pharmacological and clinical properties of previously reported agents, little was published during 1967 which constitutes an appreciable advance in this area of medicinal chemistry.

A discussion of the mechanism of general anesthesia by Wall[36] suggests that the apparent similarity of the effect of this chemically diverse group of compounds may not be a reflection of identical modes of action but rather a reflection of the varying stability of different synapses.

Krantz[37] has experimented with the intravenous use of methoxyflurane (XXVI) in corn oil emulsion for general anesthesia in animals and man. He reported rapid induction, smooth anesthesia and uneventful emergence. Among advantages cited for his procedure, particular importance to the medicinal chemist may be found in the technique of administration. This could make available for clinical trial a number of agents which owing to their high boiling point, cannot be conveniently employed by inhalation technique.



(XXVI)



(XXVII)

Ketamine hydrochloride, CI-581 (XXVII), mentioned in the two previous editions of this review continues to receive favorable clinical reports[38, 39, 40] as an injectable general anesthetic in spite of its association with hallucinations.

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Chapter 4. Analgetics --- Strong and Weak

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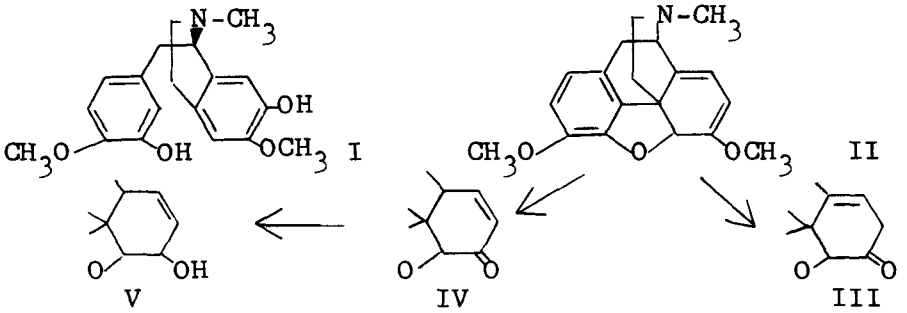
I. Introduction - The past year has been unique in recent times in that a substantial number of new analgesics have become available for general medical use in this country. These include the non-narcotic phenothiazine methotrimeprazine, the narcotic-antagonist analgesic pentazocine, the mild analgesic mefenamic acid, and the neuroleptanalgesic mixture of fentanyl and droperidol. Although synthetic work in the analgesic area has not been large, some interesting new compound classes have appeared which have both potential clinical usefulness and theoretical importance in our search for an understanding of how analgesics act.

II. Strong Analgesics

A. Morphine - Little new in the way of synthetic work was published in this area. However, Rapoport and his group[1,2] have continued their elegant work on the biosynthesis of the opium alkaloids. Earlier Barton and his colleagues[3] and Battersby and his associates[4] had put forth evidence that reticuline (I) served as a precursor of thebaine (II). This role has now been firmly established in fresh budding plants



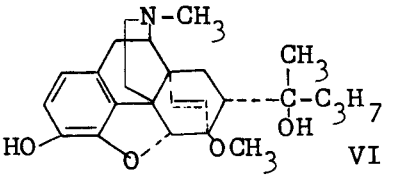
and seedlings exposed to $^{14}CO_2$ for short periods utilizing gas chromatography with simultaneous mass and activity measurements for analysis. The route to morphine is the same in seedlings as in mature plants and follows the scheme:

$$CO_2 \rightarrow \text{reticuline} \rightarrow \text{thebaine} \rightarrow \text{codeine} \rightarrow \text{morphine}$$

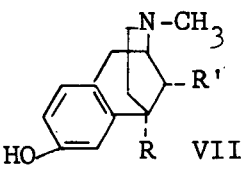
The intimate details of the conversion of thebaine to codeine has also been worked out[2,5]. Thebaine (II) is demethylated to a postulated tautomeric mixture of neopinone (III) and codeinone (IV) followed by reduction to codeine (V). Although the opium poppy is capable of demethylating codeine methyl ether, the plant apparently does not use this route and the reported isolation of this compound is attributed to the reduction of thebaine in the isolation and purification processes[6].

B. Oripavine Analogs - A classic series of papers by Bentley and his colleagues[7] describe the structure-activity relationships of a wide variety of structures derived from Diels-Alder adducts of thebaine. Among this group are found some of the most potent analgesics known. Particular attention should be directed toward the fine application of nuclear magnetic resonance spectroscopy, by Fulmor and his associates[8], to elucidate the stereochemical configurations of these interesting compounds.

The pharmacology of one of the more potent morphine-like members of this series, etorphine (M-99, VI), has recently been more extensively described by Blane and his group[9,10,11]. Depending on the test system this compound proved to be 1,000-



VI



VII

A: $R=R'=C_2H_5$

B: $R=\emptyset$, $R'=CH_3$

C: $R=C_3H_7$, $R'=CH_3$

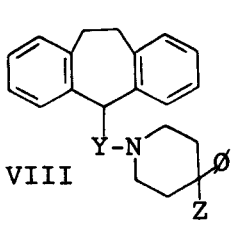
80,000 times more potent than morphine. It was qualitatively similar to morphine with the exception that low doses of etorphine did not produce excitement or emesis in dogs[9]. The distribution of etorphine in the pregnant rat was compared with that of dihydromorphine[10]. Etorphine is found to enter and concentrate more rapidly than dihydromorphine in the brains of both the mothers and the foetuses. Up to twenty minutes after intramuscular administration the concentration of etorphine was higher in the brain than in the blood. This may explain, in part, the high potency of this compound. The rapid passage of etorphine across the placental barrier and its accumulation in the fetal brain may also explain the neonatal mortality observed after large doses of this drug, a phenomenon not observed with morphine. Evidence has also been presented to show that if one avoids the sharp absorption peak of etorphine, by giving the drug sublingually, the fetal respiratory depression and lethality are much less[11].

C. Benzomorphans - Of interest is the continued finding in these compounds of a stereospecific separation between analgesic activity, as measured by the hot-plate technique, and addiction liability as assessed in the monkey. May and Eddy[12] had earlier reported the resolution of cis-5,9-diethyl-2'-hydroxy-2-methyl-6,7-benzomorphan (VIIA). The (+)-isomer had weak analgesic activity but a high physical dependence capacity (PDC). The (-)-isomer had high analgesic activity yet proved to be nalorphine-like (i.e. precipitated abstinence) in addicted monkeys. Similar findings were obtained with the 5-phenyl compound VIIB[13a]. More recently the 5-propyl-9-methyl derivative VIIC was also resolved and tested.

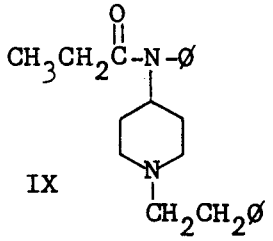
Again, the (+)-isomer is a considerably less potent analgesic than the (-)-isomer yet has a high PDC. The (-)-isomer behaves like nalorphine in addicted monkeys[13b]. The (-)-isomer of VIIA in the trans-configuration has also been prepared. It is a potent analgesic with little or no PDC[13b]. It had no antagonistic activity.

Primary addiction studies in monkeys were carried out with both (-)-cis- and (-)-trans- VIIA. The (-)-cis-compound produced few morphine-like abstinence signs on abrupt withdrawal after prolonged administration. The (-)-trans-compound produced a typical morphine-like abstinence.

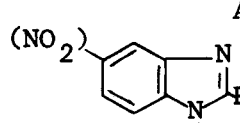
D. Miscellaneous - Davis and his colleagues[14] reported a series of amitryptyline analogs containing the normeperidine group as the basic nitrogen. Several of these had potent analgesic properties. The general structure is shown as VIII. Optimum activity was achieved when Y= $=CHCH_2-$ and Z is the



VIII



IX



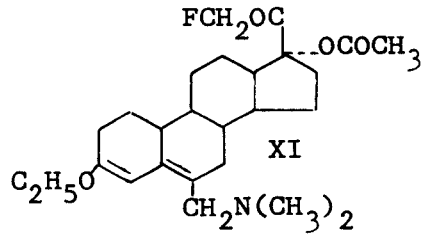
A: R=CH_2-Ø-OC_2H_5

B: R=S-Ø-OC_2H_5

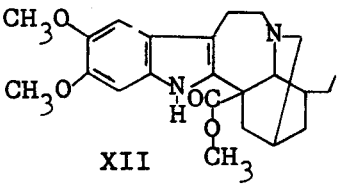
X $(CH_2)_2N(C_2H_5)_2$

reversed ester $-OCOC_2H_5$. Substituting a phenothiazine or benzhydryl for the dibenzocycloheptene system lowered activity. Also of interest in the meperidine field is the report[15] that meperidine-N-oxide is equipotent with meperidine with a longer duration of action. Fentanyl (IX) the meperidine analog in the neuroleptanalgesic mixture Innovar, was tested for analgesic activity in man and found to be 50x more potent than morphine[16].

A number of reports of new syntheses in the benzimidazole series (XA) have been reported[17-19]. As would be expected, active compounds were obtained. The necessity of the 5-nitro group for maximal activity was confirmed[17,18]. Substitution of a 2-thiophenyl (XB) also lowered activity[19].



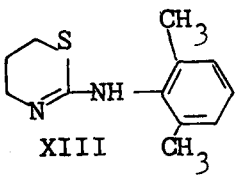
XI



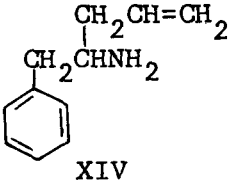
XII

A series of 16-β-amino-17α-20-dihydroxypregnanes having analgesic activity have been described[20]. A similar compound,

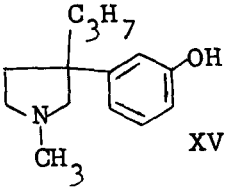
XI, was studied for addiction liability in monkeys[13b]. No suppression of abstinence was noted up to 15 mg/kg. Solubility prevented higher doses but signs of mixed stimulation and sedation were observed. An alkaloid of the voacanga



XIII



XIV



XV

series, conopharyngine (XII), was also shown to have analgesic activity[21]. The analgesia observed was unaccompanied by motor incoordination and was not antagonized by nalorphine.

The 1,3-thiazine XIII has been reported to have strong analgesic properties in animals and man[22]. The compound has a high sedative and antihypertensive component and acts like morphine on the electrically stimulated gut. Also of interest are the amphetamine derivative aletamine (XIV) and the pyrrolidine derivative, profadol (XV). Both of these compounds have been reported to have analgesic properties in animals and man although aletamine was quite weak. Profadol is currently being evaluated at Lexington for addiction liability. Aletamine had no PDC in addicted monkeys and in primary addiction studies no morphine-like abstinence was seen[13a].

E. Biochemical and Pharmacological Considerations - A number of papers appeared which implicated the central adrenergic system with analgesia. Verri and co-workers[23] reported that premedication with reserpine or α-methyltyrosine antagonized the analgesic action of morphine in mice. The central stimulating action of morphine was reduced by pretreatment with α-methyltyrosine[24]. On the other hand, Ross and Ashford[25] reported that pretreatment with reserpine inhibited the analgesic action of morphine in the tail clip test and potentiated morphine's action in the hot plate test. α-Methyldopa pretreatment prevented the inhibitory effect of reserpine on the tail clip test and showed analgesic action itself in the hot plate test. Contreras and his co-workers[26] demonstrated the analgesic activity of methyldopa and ergonovine, and that tolazoline can antagonize the analgesia produced by many narcotic analgesics. This antagonism appeared to be competitive in that the tolazoline block could be overcome by higher doses of meperidine and phenazocine. Takagi and Nakama[27] found a reduction in mouse brain norepinephrine and dopamine following the combined injection of morphine and nalorphine. Nalorphine alone had no effect on the brain content of these amines.

Morphine and related agents have been shown to cause a release of 5-hydroxytryptamine (5-HT) in the small intestine

of the dog that corresponds to the increased intestinal tone observed[28]. Further evidence for the role of 5-HT in the gastrointestinal effects of morphine were demonstrated when it was observed that methysergide, a potent inhibitor of 5-HT, antagonized the effects of morphine on gastrointestinal propulsion in mice[29].

In mice 8-azaguanine, an inhibitor of protein synthesis, antagonized the development of tolerance[30,31]. Morphine injections inhibited the incorporation of ^{14}C leucine into brain proteins. This effect of morphine could be blocked by keeping the rats at an ambient temperature of 30° and by the simultaneous injection of nalorphine[32]. The relationship of morphine's action to protein synthesis or nucleic acids is still not clear.

Mulé[33] reported that P^{32} uptake into cortical slices was stimulated by the subcutaneous administration of morphine to non-tolerant guinea pigs. Chronic morphine treatment inhibits this increased phospholid metabolism. The uptake of P^{32} into polyphosphoinositides was markedly enhanced by cortical slices from abstinent guinea pigs in the presence of morphine. Estler[34] correlated excitement produced by morphine in mice with a decrease in brain glycogen, and an increase in brain glycogen of rats sedated by an injection of morphine. Ratios of phosphorylase-a to total phosphorylase was directly related to changes in glycogen. As in other studies of this type it is difficult to ascertain whether the biochemical changes were the cause or the result of the observed pharmacological response.

A number of studies concerning the metabolic fate of morphine and its analogs have been reported. Thus, Castro and Gillette[35] studied the metabolism of ethyl morphine in male and female animals of five species. Their results suggest that there was a qualitative (Km) as well as a quantitative change (Vmax) in the drug metabolizing enzymes. A discrepancy between the *in vitro* findings of a decrease in N-demethylation of morphine and *in vivo* results has been reported by Adler[36]. These results suggest that either induction of microsomal enzymes, or no change in their synthesis occurs during morphine tolerance, rather than a decrease in microsomal enzyme synthesis as *in vitro* findings have suggested. A single injection of 30 mg/kg morphine did not inhibit synthesis of microsomal enzymes in female rats[37] and various antagonists were found to competitively inhibit demethylation of morphine and oxymorphone[38].

The non-addicting antitussive agent dextromethorphan was shown to be principally excreted in bile as d-3-hydroxy-N-methyl-morphinan and its glucuronide and sulfate conjugates[39]. Excretion dynamics following multiple doses were not significantly different than after an initial injection.

Some miscellaneous pharmacological studies were also of interest. The pharmacology of N-methylmorphine has been compared with that of morphine[40]. It was concluded that the pharmacological differences between the two compounds can be explained essentially by the inability of the quarternary salt to enter the central nervous system. Morphinone, which is proposed to have only depressant activity, and the stimulant thebaine, both decreased tidal volume but had no effect on oxygen consumption[41]. However, the compounds acted differently on seizure threshold. Morphinone raised the threshold while thebaine lowered it.

III. Narcotic Antagonists - Two recent reviews have appeared which deal with this area. One is a masterful overview of the entire field by Martin[42] while the other concerns itself to the relatively limited scope of the narcotic-antagonist analgesics[43]. This class of drugs continues to be the focus of a large research effort.

A. Pentazocine - This compound was released by the FDA in 1967. The drug is not covered by the Harrison Narcotic Act and clinical acceptane, to date, appears to be good. Laboratory and clinical studies continue to appear.

Pentazocine has been reported to be effective in relieving experimental pain in man and as a preoperative medication[44] although a high dose was associated with some incidence of psychotomimetic activity in this group of patients. The effect of pentazocine on gastrointestinal function was studied in anesthetized dogs[45]. Gastric emptying time was slowed although the pyloric sphincter was not affected at the doses used. In this regard pentazocine differs from morphine.

B. Cyclazocine - This drug continues to show promise as an adjunct in the treatment of narcotic addiction[46]. Widespread clinical trials are still in progress with continued favorable results. Mulé and Gorodetzky[47] studied the distribution and fate of cyclazocine in normal dogs, in dogs made tolerant to the drug, and in tolerant dogs after abrupt withdrawal. Recovery of free and conjugated drug in the urine and feces ranged from 40.7% in the abstinent animals to 58.5% in the tolerant dogs. The higher value in tolerant animals was due to an increased excretion of free drug in the feces. There is some indication of significant metabolism. High concentrations were found in the brain. This is similar to the finding with nalorphine and different from morphine. The failure to find significant quantities of cyclazocine in the brain of the 24-hour abstinent dog suggest that the long latency in the onset of abstinence is not due to residual quantities of drug. Finally it was found that cyclazocine disappears more slowly from the brains of tolerant animals. Tullar and his colleagues[48] reported the preparation of all

the possible stereoisomers (optical and cis-, trans-) of cyclazocine and pentazocine. They have been tested as narcotic-antagonists. In both cases the (-)-isomers were considerably more potent than the (+)-isomers. The differences between the cis- and trans- isomers were small.

C. Naloxone - This compound appears to be a pure antagonist. It has little or no analgesic activity in animals or man, does not constrict pupils or produce subjective effects and does not produce tolerance or physical dependence[49]. Naloxone can reverse the depressant effects of morphine and cyclazocine on the flexor reflex at doses which do not directly stimulate the reflex[50]. The compound has also been reported to antagonize the respiratory depressant and subjective effects of cyclazocine in man[51] and the respiratory depressant effects of pentazocine in dogs[52].

D. Miscellaneous - Wuepper and his associates[53] reported that the simultaneous administration of nalorphine or levallorphan with levorphanol resulted in a marked reduction in the concentration of levorphanol in the brain as compared to levorphanol alone. The antagonists did not affect the plasma levels of levorphanol. The combination of nalorphine with morphine did not give similar results. In 1964 Simon[54] reported that levallorphan and other synthetic analogs of morphine were potent inhibitors of bacterial growth. Greene and Magasanik[55] in a beautifully designed and carried out study, have elucidated the mechanism through which levallorphan exerts this action on Escherichia coli and HeLa cells. Levallorphan causes an immediate and marked decrease in intracellular ATP. They present evidence that suggests that the decrease in ATP concentration results from the stimulation of ATPase.

IV. Weak Analgesics - Chapter 21 on antiinflammatory drugs should also be consulted for a complete review of current research in this class of compounds.

A. Salicylates - The effects of salicylates on the gastric mucosa have been reviewed by Davenport[56]. He concluded that reabsorption of gastric secretions by the more permeable mucosal cells is more important in salicylate hypoacidity than an alteration in the secretory mechanisms. This hypothesis of the back-diffusion of gastric acid was supported by the data of Brodie and Chase[57] who demonstrated that gastric HCl but not necessarily direct contact of aspirin with the rat mucosa could cause lesions and hemorrhage. Schoenhoefer and Perry[58] have demonstrated an inhibition of the synthesis of glucosamine-6-phosphate in gastric mucosal cells of rats but not in the liver tissue of the same animals, following a single large oral dose of sodium salicylate or phenylbutazone. They have suggested that this inhibition may interfere with mucoprotein formation which in turn may be

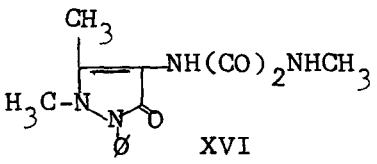
responsible for the gastric intolerance seen with these compounds. Data has been presented that indicates that salicylate is a general inhibitor of pyridine nucleotide-linked dehydrogenase enzymes in a number of in vitro preparations[59]. However, Nakaue and co-workers reported no change in chick succinic dehydrogenase activity in animals fed various levels of acetylsalicylic acid[60].

Tubular reabsorption of Na^+, Cl^-, Ca^{++}, and Mg^{++} but not K^+ was augmented by acetylsalicylic acid but not by ethoxy, acetyl or hydroxyorthobenzoic acid[61]. Brown and Hardy[62] reported that "analgesic nephritis" may be due to amidopyrine which causes papillary necrosis or phenazone which causes persistent celluria and slight kidney damage. Phenacetin was the least damaging to the kidney of the three compounds tested.

New light has been shed on the conflict over the metabolic fate of salicylamide in man. Levy and Matsuzawa[63] presented evidence that the metabolic excretion of salicylamide was predominantly in the form of a sulfate or glucuronide conjugate depending on the dose. Sulfate availability appears to be the limiting factor, i.e. at low doses salicylamide was excreted as the sulfate and at higher doses the fraction excreted as the glucuronide conjugate increased.

Dipropylacetylsalicylic acid was found to have a more intense and longer lasting analgesic activity than the same dose of acetylsalicylic acid[64]. Faye and co-workers[65] reported that 2-hydroxy-3,6-dimethylbenzoic acid and its 6-isopropyl derivative have more analgesic effect in the rat than acetylsalicylic acid.

B. Pyrazolone Derivatives - Significant analgesic activity was observed in rats following the oral administration of N-methylamide-N-(4-antipyryl)-oxamic acid (XVI) and to a lesser extent with other N-(4-antipyryl)-oxamic acid amides[66].



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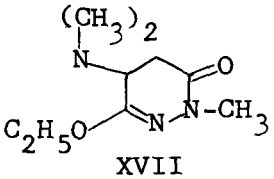
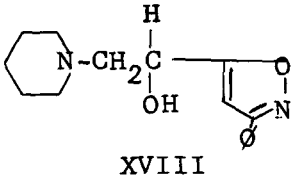


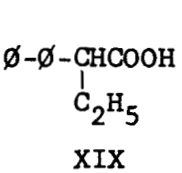
XVII



XVIII

Substitutions of homologous groups for the methylamide decreased analgesic potency until N-butylamide-N-(4-antipyryl)-oxamic acid. The N-hexamide derivative again possessed high analgesic activity. A series of pyridazinone derivatives were tested for analgesic activity in mice and 2-methyl-6-ethoxy-5-dimethylamino-3(2H)-pyridazinone (XVII) and the 4-dimethylamino analogue were found to be twice as active as aminopyrine[67]. A number of isoxazoles and related derivatives

were found to have analgesic activity[68]. The compounds with a piperidino- or morpholinoalkyl side chain were more potent. Potency was increased and toxicity decreased by oxidizing the side chain to an amino-alcohol such XVIII.



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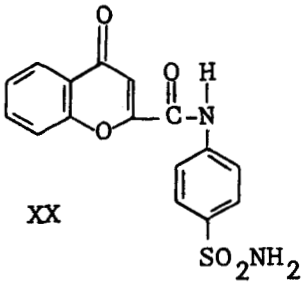

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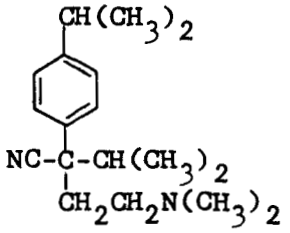

XXI

C. Miscellaneous Agents - Emele and Shanaman[69] reported that the analgesic potency in laboratory animals of the diethylaminoethanol salt of 2-(4-biphenylyl)-butyric acid (XIX) was greater than acetylsalicylic acid in three of four procedures used. A series of chromone-2-carboxamides such as XX were less toxic and more active analgesics than acetylsalicylic acid and amidopyrine in acetic acid writhing and heat-induced pain in mice[70]. A series of para-substituted phenylacetonitriles and phenylacetamides were also synthesized and tested for analgesic activity[71]. The 2-p-cumenyl-4(diethylamino)-2-isopropyl-butyronitrile (XXI) and the 2-(p-tert-butylphenyl) derivatives of the nitriles were more active in the mouse hot plate test than phenylbutazone.

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Chapter 5. Anorexigenic Agents

Frank P. Palopoli, The National Drug Company, Philadelphia, Pa.

Introduction - No breakthrough was made in the field of anorexigenic agents during 1967. With few exceptions, research continues to be concentrated on amphetamine like drugs.

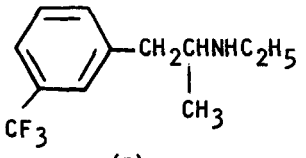
Obesity and weight control received considerable but unfavorable publicity in the lay press regarding the indiscriminate use of the so-called "rainbow pills"; the use of such preparations has been criticized.[1] Obesity, however, continues to be a problem of great concern in the United States and is considered to be a major health hazard.[2] An assessment of how anorexigenic agents are best used and the important role they play in weight control has been made.[3]

Mechanism of Action - The mechanism of action of anorexigenic agents has not been precisely demonstrated, although sufficient evidence has accumulated to fix the probable locus of their suppressant action on the lateral nuclei or feeding center of the hypothalamus. In support of earlier observations with stereotaxically induced lesions in the hypothalamus of the rat,[4] it was found that gold-thioglucose obese mice are not refractory to the anorexigenic effect of amphetamine and therefore not dependent on a ventromedial inhibitory system suggesting that the lateral hypothalamic feeding center is involved in the anorexigenic effects of amphetamine.[5] Inhibition of gold-thioglucose induced obesity by the glucose inhibitor 2-deoxy-D-glucose or its amino analogue led to the conclusion that these inhibitors interact with cells in the "satiety center"; hypothalamic cells possess a high affinity for the inhibitors since an aphagic response was not obtained with stereotaxically induced lesions in mice.[6]

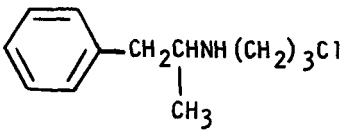
A dose-dependent increase, in electrical stimulation of the lateral hypothalamus, necessary to elicit eating in food-satiated rats, was clearly shown for amphetamine, phenmetrazine (III), methamphetamine (IVa), chlorphentermine (VIa) and diethylpropion (VII). The duration of effect also tended to be dose-dependent for the tested drugs except amphetamine and methamphetamine.[7]

A series of anorexigenic agents injected i.p. into rats 16 hrs. before sacrificing decreased hypothalamic serotonin levels. One of these, fenfluramine (I), protected mice against diarrhea and writhing induced by L-5-hydroxytryptophan. p-Chlorophenylalanine, a specific brain serotonin depletor had no effect on food consumption in normal rats and did not change the anorexigenic response of these animals to drugs. The serotonin depleting properties of these phenylalkylamines are evidently not involved in the mechanism of their anorexigenic action.[8]

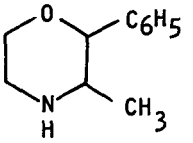
Phenalkylamine Derivatives - An examination of the recent clinical experiences with fenfluramine (I) indicates it to be an effective anorexigenic agent without cardiovascular and stimulant effects.[9,10] Its failure, at therapeutic doses, to influence the "critical flicker frequency" correlates well with the clinical observation of lack of central stimulation.[11] A sedative component is suggested since slowing of cortical waves and abolishment of cortical after-discharge were observed in cats.[12]



(I)



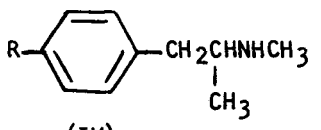
(II)



(III)

The computer technique of power spectrum analysis was used to analyze frequency changes induced by d-amphetamine and mefenorex (II) [Ro4-5282] in the EEG of the cat. Unlike amphetamine, mefenorex did not antagonize the EEG depressant effects of sodium pentobarbital in anesthetized cats, suggesting that mefenorex is less stimulating than amphetamine.[13] However, recent clinical studies report CNS stimulation in addition to the usual side effects.[14,15]

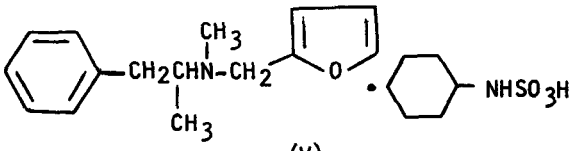
Several new derivatives of methamphetamine have been reported to be effective anorexigenic agents. An alkyl derivative (IVb) is reported to have an anorexigenic effect almost completely dissociated from the cardiovascular activity of its precursor.[16] A N-furfuryl derivative (V) [furfenorex] is reported to be effective in man,[17] and is currently being marketed in France. Its pharmacology[18] indicates it to have sympathomimetic effects in both rats and dogs.



(IV)

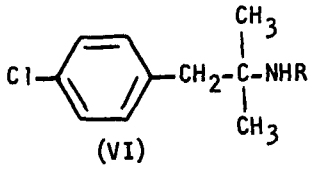
a R = H

b R = n-C_5H_{11}



(V)

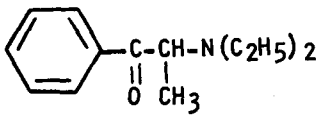
The N-ethylcarbamate of chlorphentermine (VIa) (VIb, cloforex) continues to receive attention and is reported to be an effective anorexigenic without CNS stimulation in both adults[19] and children.[20]



(VI)

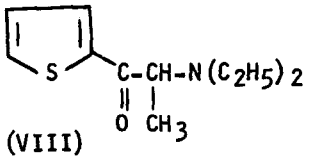
a R = H

b R = $COOC_2H_5$

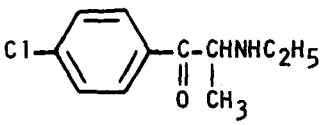


(VII)

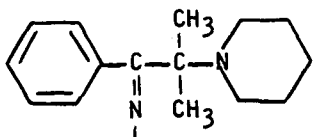
Two diethylpropion analogues (VIII) and (IX) have been described. The thiophene derivative (VIII) and related amino modifications were claimed to have anorexigenic activity comparable to amphetamine with much less CNS stimulation.[21] A chloro-substituted derivative (IX) [SKF-70948, FWH-494] was reported to be one-fifth as potent as diethylpropion in decreasing food intake in dogs; it produced less motor stimulation in the mouse than d-amphetamine, reversed and prevented reserpine induced depression. In the anesthetized cat, a biphasic blood pressure response was observed.[22] A clinical trial of (IX), at daily doses of 75-150 mg, revealed effects similar to those obtained with diethylpropion.[23] A clinical study designed to demonstrate the effect of continuous versus intermittent administration of diethylpropion was shown to favor continuous administration.[24] A related acylimino derivative (X) was reported to have one-half the anorexigenic activity of phenmetrazine in the rat, and equipotent in the dog to phenmetrazine.[25]



(VIII)

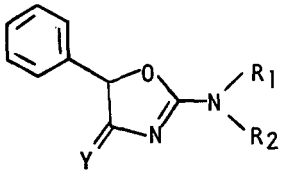


(IX)



(X)

Recent studies with 2-amino-5-phenyl-2-oxazoline (XI) [aminorex] have shown it to be an effective anorexigenic agent with no significant difference in the incidence or degree of side effects observed with marketed agents.[26,27,28]



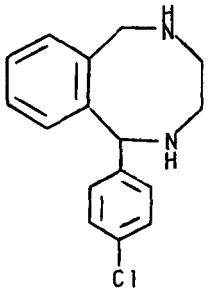
XI, Y = 2H, R_1R_2 = H, aminorex

XII, Y = O, R_1 = H, R_2 = cyclopropyl

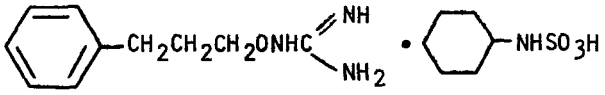
XIII, Y = O, R_1 = CH_3, R_2 = cyclopropyl

The structurally similar 2-cyclopropylamino-5-phenyl-2-oxazoline-4-one (XII) [LD-3695] is also reported to be an active anorexigen with less acute toxicity than d-amphetamine.[29] Its CNS effects are reportedly not due to MAO inhibition and it had no effect on catechol-o-methyl transferase. The N-methyl derivative (XIII) [LD-4202] is reported to be equi-anorexigenic and less stimulating than (XI).[30]

Non-Phenethylamine Derivatives - The search for compounds which bear no structural relationship to phenethylamines and have anorectic properties continues. Current studies on Wy-5244 (XIV) show it to possess only weak sympathomimetic activity in isolated cat atria and rabbit aortic strips; however, the compound apparently does alter adrenergic receptor mechanisms, since the actions of substances such as norepinephrine, d-amphetamine, phenoxybenzamine, and imipramine were profoundly influenced by Wy-5244.[31] The synthesis of (XIV) and related compounds has been described.[32]

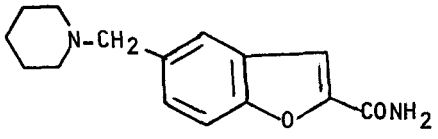


(XIV)

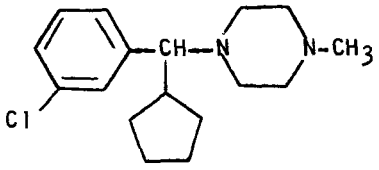


(XV)

3-(Phenylpropoxy)guanidine cyclohexane sulfamate (XV) [U-16,178F] effectively reduced food consumption in dogs, rats and mice. It was one-tenth as potent as d-amphetamine in depleting brain catecholamine levels. Unlike d-amphetamine it had no tryptamine potentiating activity and much less antireserpine activity.[33] A benzofuran derivative, (N-piperidinomethyl)-5-coumarylamide (XVI) [L-4035] has been reported to be comparable to phenmetrazine in activity.[34]



(XVI)



(XVII)

N-(α-cyclopentyl-m-chlorobenzyl)-N'-methylpiperazine (XVII) was shown to reduce food consumption in rats by 44% and caused a 19% weight loss. The compound was devoid of CNS effects.[35]

Miscellaneous - Combination therapy continues to be of interest and recent studies of anorexigenic agents with a sedative,[36] tranquilizer,[37] and a laxative,[38] have been reported. Some of these combinations did not appear to offer distinct advantages over the use of anorexigens alone. Clinical studies have been reported on the effects of anabolic[39] and thyroid analogues[40,41,42] on weight reduction. Further reports on the effects of anorexigenic agents on other metabolic factors such as oxygen consumption,[43] and plasma insulin[44] have appeared.

Methods for the detection, identification and estimation of sympathomimetic amines and their metabolites in human urine by GLC and TLC have been advanced.[45,46] The procedures of Beckett[45] lend themselves to the analysis of a wide variety of basic drugs and their metabolites. Several simple and reliable laboratory procedures have been employed to test the ability of anorexigenic or potentially anorexigenic agents to produce central stimulation in animals; these results correlate well with clinical experience.[47]

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Section II - Pharmacodynamic Agents

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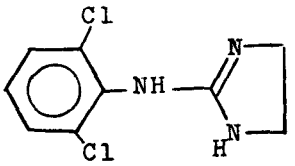
Chapter 6. Antihypertensive Agents

John G. Topliss, Schering Corporation, Bloomfield, N. J.

Catapresan® - Additional papers have appeared in the clinical literature during 1967 on this new antihypertensive agent, but little came forth in the way of new developments concerning mechanistic aspects of its action.



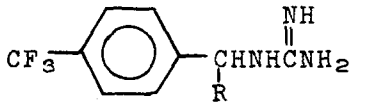
I · HCl

In one study in rats, evidence was obtained that at least part of the hypotensive action of Catapresan® (I) is due to α-adrenergic blockade.[1] The infusion of low doses (0.25 - 2.0 μg/kg) into the cerebral artery of anesthetized cats resulted in a considerable, dose-dependent decrease in blood pressure which developed suddenly, towards the end of the infusion. The same low doses, when given intravenously, had little effect on blood pressure. These results were viewed as consistent with a central mechanism of action of the drug.[2]

An investigation in 21 patients[3] produced overall results similar to those obtained previously by other investigators.[4] With a daily dose of about 500γ, there was an effective fall in systolic and diastolic blood pressure. Only a slight effect on orthostatic blood pressure was observed, and there was some slowing of the pulse rate. Sedation and dryness of the mouth were noted but were much reduced as the treatment progressed. The urinary excretion of vanilmandelic acid, the main metabolite of catecholamines, was significantly reduced on the second day of drug administration, as compared to controls. This could have been due to interference with either the synthesis or the secretion of catecholamines. The authors suggested that this observation, coupled with the initial sedative effect and the disturbed sleep of some patients, may indicate a central effect. Another trial was conducted with 25 hypertensive patients over several weeks where the desired lowering of blood pressure was attained on the fourth day of treatment. Side effects were not troublesome.[5] Renal hypertension may even respond to a measurable degree.[6] Parenteral administration of Catapresan® seems to be effective and reliable in the treatment of hypertensive crises.[7] When combined with a thiazide for chronic administra-

tion, the antihypertensive effect is equivalent to thiazide plus α-methyldopa.[8] In a clinical pharmacologic trial with 12 chronic schizophrenic patients, no antipsychotic activity was observed.[9]

Guanethidine Analogs - In a series of 25 derivatives of benzylguanidine, the most active compounds were p-trifluoromethylbenzylguanidine sulfate (II, R = H) and its α-methyl analog (II, R = CH_3). Oral doses of 30 mg/kg of the former initially increased, but subsequently decreased, blood pressure in both renal and neurogenic hypertensive dogs. Blood pressure was reduced for at least 50 hr, and the more marked reduction was observed with neurogenic hypertensive dogs. In the case of II (R = CH_3), doses of 10, 5 and 2.5 mg/kg were employed, and a good dose-response relationship was apparent. Again, the neurogenic dogs exhibited a better response than did the renal dogs. This compound was notable for its lack of side effects.[10]



II ·$1/2H_2SO_4$

Results of a study of 70 hypertensive patients treated with bethanidine for periods ranging from six months to two years have been reported. Satisfactory control of blood pressure was obtained in about three-quarters of the group, and side effects, apart from postural hypotension (38% of the patients), were rare.[11]

Liver-function test abnormalities have been noted among patients treated with guanoxan.[12]

The progress of 224 hypertensive patients treated with guanethidine for one to five and one-half years has been assessed.[13]

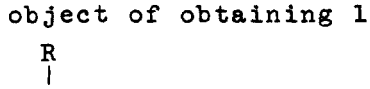
α-Methyl Dopa and Related Compounds - It has been shown by Varma[14] that α-methyldopa produces its usual antihypertensive effect in immuno-sympathectomized rats made hypertensive by metacorticoid treatment. This observation was considered to be inconsistent with the hypothesis of Day and Rand that α-methyldopa produces its antihypertensive effect by substituting a less active "false transmitter substance" for noradrenaline in the sympathetic nervous system. However, the latter authors have presented arguments to suggest that despite these findings the false transmitter hypothesis explaining the antihypertensive effect of α-methyldopa is still tenable.[15]

The proposal that a central effect might be the cause of the decrease of peripheral resistance brought about by α-methyldopa is supported by evidence that a centrally mediated hypotensive effect may be demonstrated in the cat.[16]

A number of observations have been made on the occurrence of positive Coombs tests and hemolytic anemia in patients receiving α-methyldopa.[17-20] In this connection, the nature of the α-methyldopa red-cell antibody has been investigated.[20]

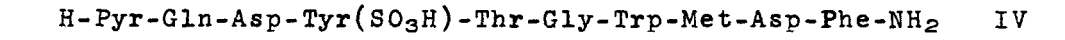
The antihypertensive effects of α-methylated catecholamine analogs have been studied.[21] In renal hypertensive rats, prolonged treatment with α-methyldopa, α-methyl-m-tyrosine, α-methyltyrosine or metaraminol produces dose-dependent decreases in blood pressure and dose-dependent depletion of myocardial catecholamines. The same order of relative activities was found in regard to noradrenaline depletion and antihypertensive effect, but there was no relationship between the degree of catecholamine depletion and the intensity of the hypotensive effect.

Peptides - A new series of peptides related to eledoisin, comprising acyl derivatives of III, has been synthesized, with the object of obtaining long-acting hypotensive drugs. Some of these compounds, in particular the butyryl and valeryl derivatives, had a significantly longer duration of action than eledoisin.[22]



III

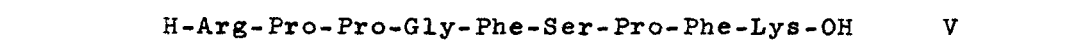
Both natural[23] and synthetic[24] forms of the decapeptide caerulein (IV) have been shown to lower blood pressure in the dog, rabbit, and in man. In the cat, the action of this substance is erratic and less intense, and in the rat it generally



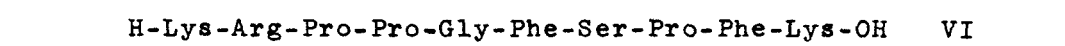
IV

causes a hypertensive or biphasic response. The duration of action, after i.v. injection in the dog, is considerably longer than for bradykinin or physalaemin. The threshold i.v. dose ranges between 0.01 and 0.1 μg/kg.[25]

Lys^{9}-bradykinin (V) is more potent than bradykinin in lowering the mean systemic arterial blood pressure of the guinea pig, but less potent in other laboratory animals. Lys^{10} Kallidin (VI) is about as active as Kallidin in this regard.[26]



V



VI

6-Glycine-8-phenyllactic acid bradykinin (VII) has been synthesized and its biological activity studied. The vasodepressive effects in the rat and rabbit and the effects on

isolated rat uterus and capillary permeability in rabbit skin were respectively 4, 2, 0.5 and 0.04 times the potency of bradykinin. In the rat, three phases of action were observed: primary hypotension, partial restoration of the arterial blood

H-Arg-Pro-Pro-Gly-Phe-Gly-Pro-PhLac-Arg-OH VII
1 2 3 4 5 6 7 8 9

pressure level, and secondary hypotension. Antibradykinin activity was not observed.[27]

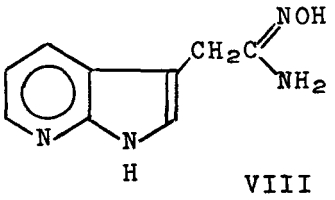
Diazoxide - An *in vitro* pharmacodynamic study was designed to specify the type(s) of inhibition involved in the vascular action of diazoxide. The results indicate that diazoxide competes with barium for a specific receptor site in the vascular smooth muscle of the rat aorta. The location of this receptor is apparently closer to the process of muscle contraction than the α-adrenergic receptor, and may be a site normally activated by calcium. The specific competitive inhibition of barium-stimulated vasoconstriction by diazoxide may help to explain the mechanism by which diazoxide, and possibly other benzothiadiazine antihypertensive agents, reduce blood pressure.[28]

From measurements of blood diazoxide levels in six adult human males at various times after i.v. drug administration, the half-life of diazoxide in blood was determined as 28.0± 8.3 hr.[29]

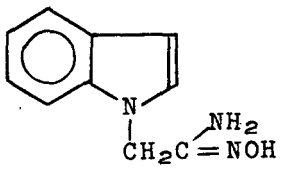
Preliminary studies have indicated that a combination of diazoxide and furosemide may be of value in the treatment of the hypertensive uremic patient.[30]

Diuretics - The antihypertensive properties of single doses of furosemide were evaluated in 113 patients and it was found that doses over 120 mg consistently produced a fall in arterial pressure, whereas smaller doses did not. The antihypertensive effect of high doses of furosemide did not seem to be related to the diuretic effect or to the decrease in plasma volume.[31]

Other Antihypertensive Agents - 7-Azaindole-3-acetamidoxime (VIII) and indole-1-acetamidoxime (IX) exhibited antihypertensive properties in the renal hypertensive rat and dog.[32,33] The ED_{50} values for reduction of blood pressure to normotensive levels following oral administration to hypertensive rats were 14.2± 3.1 and 18.2± 4.5 mg/kg for VIII and IX respectively. Oral administration of either VIII or IX, once daily, to the hypertensive dog, produced gradual, sustained lowering of blood pressure without side effects. The maximum lowering of blood pressure occurred at three days. Neither compound lowered the



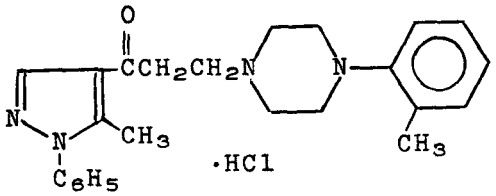
VIII



IX

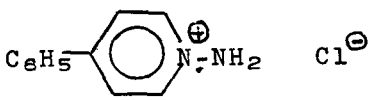
blood pressure of the normotensive rat or dog. Additional studies suggest that the antihypertensive activity of VIII may be due to catecholamine release and depletion without the bretylium-like component of action seen with guanethidine. Some aspects of the effect of IX suggest catecholamine release and α-adrenergic blocking activity, but these properties do not appear sufficient to account for the antihypertensive pattern of action seen in the hypertensive dog.

The antihypertensive properties of 1-(5-methyl-1-phenyl-4-pyrazolyl)-3-[4-(o-tolyl)-piperazinyl]-1-propanone hydrochloride (XI) have been studied in animals.[34] It produced a prolonged fall of blood pressure when administered i.v. or i.p. to anesthetized cats and dogs and lowered the blood pressure of renal hypertensive rats to normotensive levels. The compound did not show any ganglionic blocking activity or marked interference with the transmission of impulses in the cervical sympathetic chain. A significant depletion of catecholamines occurred from rat heart and brain, and the compound was also shown to interfere with the uptake of noradrenaline by the rat heart. In addition, it produced marked peripheral vasodilation, possibly by acting on adrenergic β-receptors or by sensitizing these receptors to the action of adrenaline.



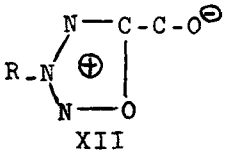
X

Pharmacological investigations of 1-amino-4-phenyl-pyridinium chloride (XI) have suggested that the compound may be a useful antihypertensive agent.[35,36] The compound depletes peripheral but not central stores of noradrenaline in the mouse and rat. The effects on the blood pressure of anesthetized animals are similar to those produced by guanethidine. However, in contrast to the latter compound, it does not cause adrenergic blockade. Daily oral



XI

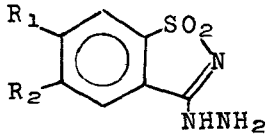
administration of XI in doses of 5 mg/kg to renal hypertensive dogs caused a pressor response on the first day (not seen if smaller initial doses were used), but on the second and third days there was a gradual reduction in the mean arterial blood pressure of 30 - 40 mm. mercury. The antihypertensive action of the compound is probably related to its action on stores of catecholamine in sympathetic nerve endings.



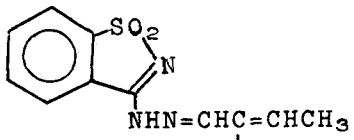
XII

A number of alkyl-substituted, mesoionic ψ-oxatriazoles (XII) were found to produce, in anesthetized dogs, a rapid, deep, and sustained hypotension with no observable side effects.[37] The potency followed a reverse order of mesomeric contribution of the alkyl substituents. Molecular orbital calculations indicated a large positive character on N_3, which, when compared with the corresponding 3-alkylsydnone and 4-acylsydnone nitrogen atom, seems to correlate with hypotensive potency.

Some 3-hydrazino-1,2-benzisothiazole 1,1-dioxides (XIII) and various related hydrazones, carbamides, sulfamides, pyrazoles and pyrazolines have been synthesized and their hypotensive activities evaluated orally in the Goldblatt rat preparation.[38-40] A large number of these had better than



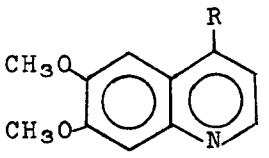
XIII



XIV

minimal activity, with some showing a pronounced blood pressure lowering effect. One member of the series, 3-[2-(2-methyl-2-butylidene)hydrazino]-1,2-benzisothiazole 1,1-dioxide (XIV), has undergone extensive pharmacological studies, which indicate that it probably has a mechanism of action other than blockade of the central or peripheral nervous systems.

The quinoline derivatives XV - XVII were found to exhibit marked hypotensive activity in anesthetized rats and dogs, apparently by mechanisms involving α-adrenergic blockade and direct musculotropic depression. In addition, it appears that XV and XVI lower blood pressure via a central mechanism. Based on data obtained in the anesthetized rat, XVI was the most potent of the three compounds.[41]

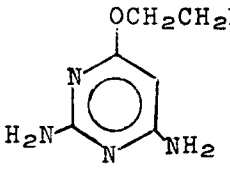


XV; R = -N=CH-C_6H_3(3,4diMeO)

XVI; R = NH_2

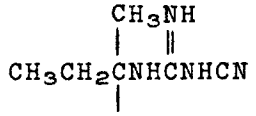
XVII; R = OH

2,6-Diamino-4-[2-(dimethylamino)ethoxy]pyrimidine (XVIII) produced a hypotensive response in anesthetized dogs, cats and rabbits, and in unanesthetized neurogenic hypertensive, renal hypertensive, and normotensive dogs. The lowered blood pressure was ascribed to direct smooth muscle relaxation as evidenced by a fall in peripheral resistance in the perfused dog hind limb and by relaxation of isolated vascular smooth muscle. This action is unrelated to occupation or blockade of adrenergic or other types of receptors.[42]



XVIII

Guancydine, 1-cyano-3-t-amylguanidine (XIX), exerted a hypotensive effect in renal hypertensive dogs, but not in neurogenic hypertensive or normotensive dogs, Repeated oral doses of 20 mg/kg over a 12 day period to metacorticoid hypertensive rats brought about a reduction in both blood pressure and cardiac hypertrophy. The hypotensive effect occurred by a mechanism other than α or β-adrenergic blockade, adrenergic neurone blockade, ganglionic blockade or histamine liberation.[43] Oral doses of guancydine in rats were found to reduce the pressor response to i.v. administered angiotensin.[44]



XIX

General - A double-blind study was conducted in which 40 patients with moderate or severe hypertension were treated with α-methyldopa, guanethidine, guanoxan and guanoclor. The last named drug had to be excluded from the trial because of severe side effects occurring after its administration. The results indicated that, where a satisfactory response is not obtained with diuretic therapy, the drug of choice to be added is α-methyldopa. When the latter does not have the desired effect, a sympatholytic agent is then given, and for this purpose the results showed that guanoxan has an advantage over guanethidine.[45]

A study was conducted on the possible resetting of "barostats" in hypertensive patients, which suggests that in some patients the barostat may, in fact, be reset. It was further apparent that the initial level of pressure and extent of vascular complications are more important factors than the duration of high blood pressure in determining which patients fall into this group.[46]

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Chapter 7. Diuretic Agents

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Although new types of diuretics have contributed greatly to the progress of renal physiology in recent years, the biochemical mechanisms underlying the diuretic-saluretic actions of these agents are not yet well understood. However, the rapidly increasing understanding of hormonal mechanisms which control metabolic pathways involved in ion transport and water permeability through epithelial structures, including those of the kidney, promises significant mechanistic progress in the future. Recent biochemical studies related to the mechanism of action of these drugs are summarized in this report, along with the major chemical, pharmacological and clinical developments published in the field of diuretics during the past year.

Reviews - Current views on the concepts of diuretic therapy,[1] the pharmacology of the established diuretic drugs,[2] the regulation of renal water metabolism,[3] and the effects and clinical significance of electrolyte disturbances on renal function[4] were discussed in informative summaries published in 1967.

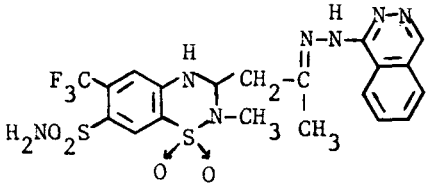
The diuretic activity and pharmacological properties of the organic mercurials and of ethacrynic acid and furosemide are already well-documented. Discussion of these agents will be confined to recent studies pertaining to their biochemical mechanisms of action.

Organic Mercurials - The high Na^+, K^+-activated adenosine triphosphatase (ATPase) activity occurring in membrane fractions of renal tissue, and the ability of organic mercurials to inhibit renal ATPase *in vitro* and *in vivo*, have led to suggestions that this may be the basis for their diuretic action.[5,6] Supporting this hypothesis is the recent observation that the effects of ouabain, an established ATPase inhibitor known to inhibit net sodium reabsorption when injected into the renal artery of the dog, are diminished or decreased in animals pretreated with the diuretics mercaptomerin or chlormerodrin.[7] Inhibition of ATPase by these agents appears to be the result of non-competitive blockade of its K^+ binding site, in contrast to inhibition by ouabain, which is reversed by K^+. However, in spite of this and other positive evidence, not all data are consistent with the above concept. The contradictory observations have been summarized and in part rationalized by Nechay *et al.*[7], who conclude that the mechanism of action of the organic mercurials is not yet clearly established.

Ethacrynic Acid - A quantitative correlation between inhibition of guinea pig Na^+, K^+-activated membrane ATPase *in vitro* and the pharmacological effects of ethacrynic acid in the dog has been established by Duggan and Noll.[8] Their observation that rat membrane fractions are insensitive to inhibition unless pretreated with a detergent for the removal of endogenous cations, seems to explain why ethacrynic acid is not diuretic in the rat.[9] The finding that ouabain fails to increase sodium excretion to the usual extent

in dogs pretreated with ethacrynic acid is also consistent with the assumption of a causal relationship between ATPase inhibition and diuretic action.[7] However, in view of a number of reports that conflict with this interpretation,[7,8,10] the mechanism of action of ethacrynic acid requires further study. It would be of interest, for example, to know what effects ethacrynic acid has on sulfhydryl-containing enzyme systems, other than ATPase, in vivo.

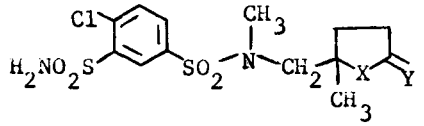
Furosemide - Furosemide, which is natriuretic and diuretic in the rat, inhibits kidney membrane ATPase in that species.[10] It decreases short-circuit current and potential difference in the toad bladder;[11] these effects are reversed by vasopressin. The failure of Ex 4847, a competitive antagonist of the diuretic action of hydrochlorothiazide, to reduce the diuretic response of furosemide suggests that hydrochlorothiazide and furosemide differ in mechanism of action.[12] This interpretation is supported by the observation that natriuresis in potassium-deficient rats is increased with furosemide, but decreased with hydrochlorothiazide.[13] Intravenous administration of furosemide to rats results in a decrease in the renal concentration of reduced glutathione, which appears inversely related to drug blood levels and degree of sodium saluresis, suggesting that furosemide inhibits glutathione reductase.[14] Since a decrease in intracellular levels of reduced glutathione in isolated bovine lens is accompanied by inhibition of ion transport, the decrease of reduced glutathione in the kidney may also be related to inhibition of ion transport and, hence, relevant to the mechanism of action of furosemide.[14,15]



Ex 4847

Mefruside - Pharmacological[16-19] and metabolism[18,20] studies of mefruside (I), a new, clinically useful sulfonamide diuretic, briefly mentioned in the previous review, have been reported. Mefruside belongs to the class of benzene-1,3-disulfonamides of the chlorphenamide type, which has thus far received relatively little attention. It differs from chlorphenamide, however, in that it does not increase bicarbonate excretion at therapeutic doses, but predominantly promotes the excretion of water and sodium accompanied by chloride.

The diuretic action of mefruside is believed to be mediated by the lactone (II) (or its corresponding hydroxycarboxylic acid), which has been isolated as the major metabolite from rat, dog and man.



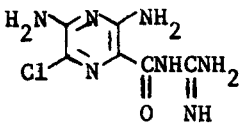
I X = O; Y = H_2 III X = NCH_3; Y = O
II X = O; Y = O IV X = NC_2H_5; Y = O

Structure-activity relationship studies reveal that compounds in which the tetrahydrofuranyl substituent is replaced by the N-alkylpyrrolidonyl moiety (III,IV) are as

potent as mefruside in rats. Interestingly, the (-) enantiomers of mefruside and its lactone are several-fold more potent than the (+) antipodes.[17]

Like furosemide, mefruside does not affect glomerular filtration rate. Inhibition of sodium and water reabsorption appears to be confined to the loop of Henle, as is the case with hydrochlorothiazide.[16,21] The drug lowers the blood pressure of Grollman rats in a dose-dependent fashion, and appears to have antihypertensive properties in man. The clinical dose range as a diuretic is 0.08 to 1.2 mg/kg.[19] It seems that mefruside, although differing in certain pharmacological details, is related in its profile of diuretic activity to agents like hydrochlorothiazide and chlorthalidone.

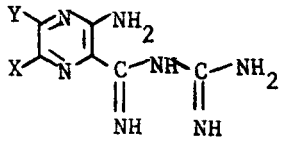
Pyrazines and Pyrimidines - The natriuretic and potassium-conserving effects of amiloride (Colectril®) in rats and dogs have now been described in detail.[22] The drug moderately increases sodium elimination, accompanied by bicarbonate and to a lesser extent chloride, and decreases potassium excretion; water diuresis is not pronounced. Amiloride is excreted unchanged by rat, dog and man. It increases the potential difference and the short circuit current of the isolated ventral skin of the frog and reverses the effects of vasopressin in this preparation.[23] In this and other pharmacological respects, it resembles triamterene.



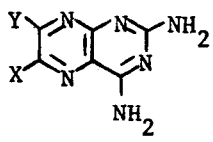
amiloride (Colectril®)

The electrolyte excretion pattern has been essentially confirmed in clinical situations.[24-31] The natriuretic and antikaliuretic effects of a single oral dose of 20 mg persist for approximately 20 hours.[24] Amiloride is especially useful in combination with other diuretics in patients with refractory edema or cirrhosis with ascites, and in hypokalemic conditions resulting from the administration of these drugs. Although apparently not itself possessing the hyperglycemic and hyperuricemic characteristics of the thiazides, amiloride does not prevent increases in fasting blood sugar and uric acid concentration induced by hydrochlorothiazide.[29] Marked increases in aldosterone secretion with lesser rises in plasma renin have been observed following amiloride administration, presumably the result of potassium retention and renin activation consequent to natriuresis.[31]

A variety of analogs of amiloride have been reported, but they are generally less potent than many of the previously described compounds in reversing the electrolyte excretion effects in deoxycorticosterone acetate-treated adrenalectomized rats.[32,33] Cyclization of a series of N-amidinopyrazinecarboxamidines (V) has provided a number of new 2,4-diaminopteridines (VI, X = Cl; Y = NH_2, $N(CH_3)_2$, $N(C_2H_5)_2$, $NHCH(CH_3)_2$) similar in structure and activity to triamterene (VI, X = Ø; Y = NH_2).[34] A direct comparison of appropriate derivatives of the different series indicates that the order of potency is pyrazinecarboxamide > pteridine > pyrazinecarboxamidine.[34]

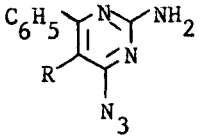


V



VI

Since the natriuretic and antikaliuretic actions of amiloride and triamterene are not dependent upon the presence of aldosterone, the biochemical alterations caused by these drugs should be elicited not only in intact, but also in adrenalectomized animals.[22,35] Enzymatic changes following administration of amiloride and triamterene to intact and adrenalectomized rats have been reported by Senft.[14] Both agents reduce the activities of renal α-ketoglutarate dehydrogenase and the pyruvate dehydrogenase complex. Noncompetitive inhibition of the pyruvate dehydrogenase complex by amiloride was also demonstrated in vitro. Whether these enzymatic changes are linked to metabolic pathways involved in tubular Na^+ and K^+ transport, and thus are relevant to the modes of action of triamterene and amiloride, is not known. In this connection, attention is drawn to earlier reports which indicate that pretreatment of rats with 6-aminonicotinamide abolished the expected natriuresis and decreased the kaliuretic effect of triamterene,[37] as well as to evidence suggesting that triamterene is capable of forming false nucleotides.[38]

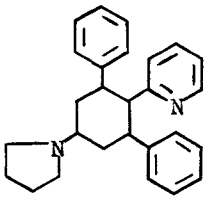


R = $-CH_2C{\equiv}CH$ (SC-16100)
$-CH_2CH_2OC_2H_5$ (SC-16102)

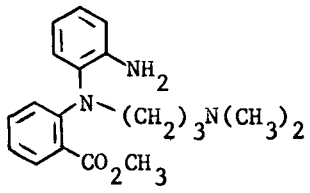
The azidopyrimidines SC-16100 and SC-16102 have been previously described as non-specific antagonists of vasopressin. The method used to screen diuretics for such activity has been reported in detail.[39] SC-16102, as well as some analogous azidopyrimidines, have also been studied in intact and adrenalectomized deoxycorticosterone acetate-treated rats.[40] SC-16102, the most potent derivative, has been found to be approximately twice as potent as hydrochlorothiazide on the basis of urine output and natriuresis. Kaliuresis, however, is the same as that of hydrochlorothiazide, which suggests a more favorable Na/K excretion ratio.[41]

Other Diuretic Agents - A new type of non-kaliuretic diuretic is 1-(N-pyrrolidino)-3,5-diphenyl-4-(α-pyridyl)-cyclohexane (Su-15049) synthesized by Robison et al. during chemical studies related to the alkaloid lobilane. Su-15049 possesses marked dose-dependent diuretic, natriuretic and chloruretic activity in the rat and dog by oral administration with only a slight, inconsistent effect on potassium excretion.[42] Although activity is dependent on the presence of the adrenals, Su-15049 is not an aldosterone antagonist; rather, an essential requirement for a natriuretic response seems to be the presence of modest amounts of glucocorticoids.[43] Glomerular filtration rate is not appreciably affected by Su-15049. Stop-flow studies

indicate that its site of action is the proximal portion of the nephron and, possibly, the distal ion exchange mechanism.[44] Administered concomitantly with hydrochlorothiazide to dogs, it caused an augmented diuretic, natriuretic and chloruretic response and decreased the kaliuresis induced by the thiazide.[45]



Su-15049



VII

Methyl N-(o-aminophenyl)-N-(3-dimethylaminopropyl)-anthranilate (VII) is said to be an orally effective diuretic agent in the rat and dog, but no detailed data have been reported.[46]

Diuretic-Induced Disturbances of Carbohydrate Metabolism - The hyperglycemic and potential diabetogenic effect of the benzothiadiazine group of diuretics is well-known. While some authors have considered this to be an inherent property of the thiazide structure, others have related it to the kaliuretic potency of these agents. A series of recent reports by Senft and coworkers provides valuable insight into this problem. According to their studies, hypokalemia is largely, but not exclusively, responsible for thiazide-induced glucose intolerance, and evidence has been obtained to show that thiazides can cause hyperglycemia independent of K^+ loss. It has been suggested that the biochemical mechanisms underlying this K^+-independent component of hyperglycemia involve (a) inhibition of cyclic 3',5'-AMP phosphodiesterase, (b) adrenal discharge, and (c) inhibition of glycogen synthetase as the result of increased intracellular levels of cyclic 3',5'-AMP.[14,47-49]

The alterations of carbohydrate metabolism caused by K^+ loss have been attributed to (1) an increased rate of glycogenolysis,[48,50] (2) enhanced gluconeogenesis,[51] and (3) reduced cellular glucose transport and uptake,[51] on the basis of evidence which includes the following observations: Negative K^+ balance results not only in decreased extracellular K^+ concentration, but also in diminution of cellular K^+ in skeletal muscle.[51] This cellular deficit is partially compensated for by an increase in intracellular Na^+ concentration. Na^+ inhibits glycogen phosphorylase phosphatase, an enzyme catalyzing the reconversion of active glycogen phosphorylase *a* to its inactive *b*-form. Hydrochlorothiazide, which increases intracellular Na^+ in skeletal muscle, is thus capable of accelerating the rate of glycogenolysis not only by inhibition of cyclic 3',5'-AMP phosphodiesterase and adrenal discharge (see above), but also by inhibition of glycogen phosphorylase phosphatase. Enhancement of gluconeogenesis (increased activity of glucose-6-phosphatase) and reduction of basal cellular glucose transport and uptake has been related to increased glucocorticoid secretion, since the cortico-

sterone content of the adrenals and glucose-6-phosphatase activity in liver and kidney of rats increased during K^+ deficiency (corticoids are known to induce glucose-6-phosphatase).[51]

It is now rather well established that diazoxide inhibits insulin secretion in animals and man.[50,52-55] Chronic administration of hydrochlorothiazide and furosemide to rats did not, however, adversely effect insulin secretion in response to an elevation of blood glucose.[47,49] These findings could explain the observation that hyperglycemia is most frequently aggravated or precipitated in diabetic patients, controlled either by diet or with sulfonylureas, who cannot respond to an increase in blood glucose with the pancreatic release of insulin.[47]

Hyperglycemia induced by non-thiazide diuretics has also been described occasionally.[56-61] The potassium-sparing diuretics, however, seem not to have been directly implicated in disturbances of carbohydrate metabolism. Recent comparative studies demonstrate that not only ethacrynic acid, furosemide and the recently introduced mefruside, but also potassium-sparing triamterene have hyperglycemic properties in rats.[62,63] Acetazolamide, mersalyl and spironolactone did not produce acute hyperglycemic effects in these studies.[62] Ethacrynic acid also causes hyperglycemia in mice, in which species, in contrast to the rat, it is diuretic.[64] Daily cortisone treatment failed to restore the hyperglycemic response of ethacrynic acid in adrenalectomized animals. This observation is in contrast to what has been reported for diazoxide, and suggests a dissimilarity between benzothiadiazine- and ethacrynic acid-induced hyperglycemia.[60,64]

Mechanisms of Renal Water Reabsorption - Much experimental evidence supports the hypothesis, first advanced by Orloff and Handler, that cyclic 3',5'-AMP is responsible for the increase in sodium transport and osmotic water flow that characterizes the physiological response to vasopressin.[65] In a recent study, Chase and Aurbach have identified two distinct adenyl cyclase systems located in renal membrane fractions of rats, one in the cortex and the other in the medulla.[66] While the medullary enzyme was stimulated by vasopressin, cortex adenyl cyclase was more responsive to parathyroid hormone than vasopressin. The localization of parathyroid-sensitive adenyl cyclase in renal cortex and vasopressin-sensitive adenyl cyclase in renal medulla appears to be consistent with reports that cellular transfer of calcium and phosphate occurs primarily in the proximal portions of the nephron, and sodium transport and water permeability are stimulated by vasopressin mainly in the collecting tubules. Vasopressin-induced stimulation of adenyl cyclase in the cortex may reflect its additional action on the distal tubule.[66]

In this regard, it is worth mentioning the evidence that cyclic 3',5'-AMP is generated at two separate sites in the cells of the toad bladder. Petersen and Edelman,[67] observed that an increase in the concentration of calcium in the bathing medium reduces the hydro-osmotic effect of vasopressin without altering its capacity to stimulate sodium transport. They proposed that cyclic 3',5'-AMP formed at one site is responsible for sodium transport, while that formed at the other site regulates water movement. Orloff and Handler have

confirmed these studies and noted additionally that calcium interferes with the hydro-osmotic effect of theophylline.[65] If these observations have relevance to the kidney, they suggest that parathyroid hormone, by altering cellular calcium transfer, modulates the effect of vasopressin-induced tubular water reabsorption. Interestingly, Senft has recently rationalized the reabsorption of water in the cortical segments of the kidney on the basis of altering concentrations of 3',5'-AMP in response to changing vasopressin secretion. However, he concluded that in the more distal parts of the nephron, increased water reabsorption is relatively more dependent upon the trans-tubular osmotic difference.[14]

Finally, Handler et al. have reported evidence that implicates the catecholamines as regulators of vasopressin action.[68] Epinephrine reduces the response of the toad bladder to vasopressin. Blockade of this effect by phenoxybenzamine, suggests that α-adrenergic stimulation underlies the suppressive effect of epinephrine. The permeability changes caused by theophylline, epinephrine and adrenergic blocking agents in the toad bladder have been related by Turtle and Kipnis to alterations in the intracellular concentrations of cyclic 3',5'-AMP.[69] As in isolated pancreatic islet cells and fat cells, α-adrenergic receptor stimulation decreases cyclic nucleotide synthesis in the toad bladder.

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Chapter 8 Angina Pectoris and Antianginal Agents

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> "Our knowledge then of angina pectoris stops short of its symptoms. The idea of it cannot be made to rest in any definite form of disease beyond them. We are sure of what it is as an assemblage of symptoms. We are not sure of what it is as a disease." Latham on Angina Pectoris (1847)[1]

At times, our present understanding of angina pectoris seems as imperfect as it was in 1847. Treatment of angina is frequently based on wishful thinking rather than any therapeutic rationale. General belief holds that angina pectoris is a result of myocardial ischemia, usually in the presence of intraluminal coronary artery disease or disease causing cardiac hypertrophy, and evoked by disturbances in the balance of myocardial oxygen supply and demand. However, the possibility of a non-arteriosclerotic cause of angina pectoris cannot be excluded. Post-mortem[2,3] and coronary arteriographic studies[4] have shown that 15 to 38% of anginal patients have no discernible coronary artery disease. Reflex coronary artery spasm has been indicted as a possible cause of anginal attacks and myocardial infarcts. Arguments for the reflex spasm theory are based on angiographic observations of spasm in humans and the induction of coronary artery spasm in experimental animals.[5]

Angina pectoris is an extremely difficult syndrome to study. Clinical studies of antianginal agents are hampered by a high placebo effect and a 50% spontaneous remission rate among anginal patients.[6] As a result of these difficulties, the significance of uncontrolled or poorly designed clinical reports must be questioned. The use of the ECG in diagnosing angina pectoris has been improved by recent studies of telemetered ECG during exercise[7,8] and atrial pacing techniques.[9] Sowton was able to induce anginal attacks by atrial pacing at 120-150 beats per minute.[10] Elevation of left ventricular pressure has been observed during anginal episodes and is regarded as evidence of left ventricular failure.[11,12] The assessment of angina pectoris and myocardial ischemia has been facilitated by recent studies of catecholamine excretion,[13] serum enzymes,[14,15] antiheart antibodies,[16] and myocardial serum lactic acid levels and arteriography.[17]

Traditional beliefs of relieving or preventing anginal pain by increasing coronary blood flow (CBF) are giving way to a new understanding of angina pectoris based on myocardial metabolism, changes in segmental resistance, myocardial collateral circulation, autoregulation of CBF, and myocardial sympathetic innervation. The improvement in our understanding of angina pectoris is, in part, due to new experimental methods of evaluating antianginal agents.[18] These methods include new tracer techniques,[19] ameroid constrictors,[20] oxygen electrodes,[21] flowmeter catheters,[22,23]

strain gauge catheters,[24] and new procedures for inducing infarcts.[25]

The use of coronary vasodilators in treating angina pectoris or myocardial ischemia has been rather disappointing. As a result, there has been an interest in the non-drug treatment of angina based on surgical revascularization,[26-29] or exercise stress.[30] Carotid sinus nerve stimulation reduces myocardial oxygen requirements by lowering arterial blood pressure, heart rate, and myocardial contractility. Stimulation of the carotid sinus nerve by means of an implanted radiofrequency stimulating unit has been recently used in the treatment of essential hypertension.[31] Activation of similar units in anginal patients is reported to abolish pain within ten seconds and to prevent angina during exercise.[32] Carotid sinus nerve stimulation is not without risk, as it may produce cardiac standstill or arrhythmias. The problem of arrhythmias is particularly severe in patients suffering from recent myocardial infarction. The use of implanted stimulators will undoubtedly gain greater acceptance in anginal therapy.

The mechanism by which coronary vessels respond to metabolic requirements is of prime importance. The normal myocardium possesses a relatively uniform tissue blood flow, while flow through the diseased heart is heterogeneous.[33] As our understanding of the autoregulatory mechanism improves, it becomes more apparent why coronary vasodilators have uniformly failed to improve the anginal patient. An understanding of the autoregulatory process also explains previous observations that coronary vasodilators may increase CBF in normal individuals yet decrease CBF in anginal patients.[21] The ideal antianginal agent should improve oxygenation and nutrition of ischemic tissue without upsetting the autoregulatory mechanism of the normal myocardium. Thus, improvements in total CBF or enhanced oxygen content of coronary sinus blood have little relevance to the therapy of the ischemic heart. The autoregulatory mechanism of adjusting regional CBF has been described in terms of ATP and its breakdown products,[34,35] plasma proteins,[36,37] catecholamines,[38] and myogenic elasticity.[39] The coronary circulation and various factors implicated in its regulation has been recently summarized in an excellent review by Schaper.[40]

In the treatment of a disorder as diverse as angina pectoris, one would expect to find a wide variety of agents promoted as beneficial to the anginal patient.[41,42] Traditionally, drugs are claimed to improve angina pectoris on the basis of increased CBF, improved myocardial nutrition, decreased cardiac energy expenditure, or improvements in myocardial performance as a result of interference with cardiac sympathetic innervation.

Amiodarone (I) is currently undergoing clinical investigation in Europe as a specific coronary vasodilator which is eight times as active as amplivix.[43,44] Amiodarone is claimed to improve myocardial nutrition in rats and dogs by reducing catecholamine-induced ATP waste.[45,46] Charlier claims adrenolytic activity for amiodarone and suggests that it reduces cardiac work without reducing cardiac output and reduces myocardial oxygen consumption and coronary artery resistance.[47]

Visnadine (II) is a spasmolytic vasodilator which is reported to be

ten times as active as khellin. In man, visnadine produces no systemic effects on blood pressure, respiration, or peripheral vasodilation. Uncontrolled studies have claimed visnadine to be beneficial in angina pectoris and myocardial infarction.[48,49] Visnadine has a positive inotropic action and increases myocardial minute volume,[50] effects which would seem to contraindicate its use in angina pectoris. Visnadine bears resemblance to khellin, a drug which has been shown to have no value in anginal therapy.[51] Unless carefully controlled studies show otherwise, Visnadine would appear to have limited usefulness in angina pectoris.

Intraveneous administration of carbochromene (III) produces a decrease in myocardial arterio-venous oxygen difference (AVO_2D). The effect of carbochromene (2 mg/kg) on AVO_2D was abolished by pretreatment with reserpine, while the effect of higher doses (of carbochromene) was enhanced.[52] High oral doses (of carbochromene) have been reported to produce little or no increase in CBF in conscious dogs with chronically implanted flowmeters.[53] Clinical studies have shown that carbochromene produces an increase in CBF in normal subjects. However, when carbochromene was given to patients with coronary artery disease there was a 10% decrease in CBF.[54] In a double blind study of 41 patients, carbochromene improved subjective responses, such as the severity of anginal attacks and exercise tolerance. Carbochromene's effect on the number of anginal attacks and nitroglycerin consumption was indistinguishable from placebo. Carbochromene produced joint pain in a number of patients.[55] Although the exact mechanism of carbochromene's action is not known, it has been proposed that it prolongs the action of adenosine on coronary arteries[56] or that it interacts with beta-adrenergic receptors.[40]

Pyridinolcarbamate (IV) is a bradykinin antagonist which has been found to be anti-arteriosclerotic in rabbits. Initial reports have claimed pyridinolcarbamate to be effective in angina pectoris and in opening occluded arteries in arteriosclerotic patients.[57,58] Pyridinolcarbamate has no effect on serum or liver cholesterol levels in rats. Acetate($1\text{-}C^{14}$) incorporation into cholesterol is inhibited, and there is no effect on the incorporation of mevalonic acid($2\text{-}C^{14}$).[59] Pyridinolcarbamate has no effect on the incidence or extent of aortic or coronary arteriosclerosis in monkeys maintained on high fat diets.[60] Little or no anti-bradykinin activity was found in a recent survey of 127 pyridinolcarbamate analogues.[61]

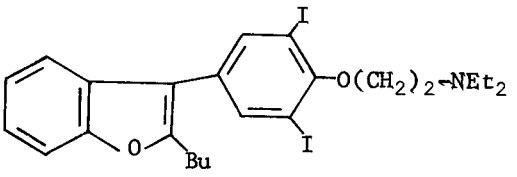
Lidoflazine (V) is a long-acting coronary vasodilator which produces a 2-6 fold increase in CBF and increases myocardial $P\text{-}O_2$.[62] In high doses, lidoflazine causes peripheral and cerebral vasodilation. In anesthetized dogs, lidoflazine produces an increased heart rate, decreased mean systemic pressure, small increases in myocardial oxygen consumption, and a decrease in AVO_2D. Cardiac output is increased, as is left ventricular work.[63] Lidoflazine's effect on CBF is similar to that produced by dipyridamole (VI). Recent work has shown that, like dipyridamole, lidoflazine potentiates adenosine, either by interfering with its uptake by red blood cells or by direct inhibition of adenosine deaminase.[64] Except for its coronary dilating activity, lidoflazine seems to produce the opposite changes from clinically-accepted antianginal agents. Most antianginal agents lower

myocardial energy requirements by reducing cardiac output and left ventricular work. An agent which increases cardiac output and left ventricular work may promote anginal attacks by increasing myocardial oxygen requirements.

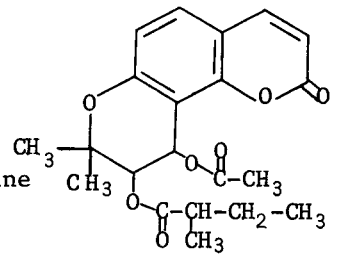
Lidoflazine, dipyridamole, carbochromene, prenylamine (VII), iproveratril (VIII), and nitroglycerin were recently studied in conscious, trained dogs with chronically implanted flowmeters. Lidoflazine, dipyridamole, and nitroglycerin were orally active coronary dilators. Carbochromene, iproveratril, and prenylamine showed only marginal activity at high dose.[53]

In recent years there has been a good deal of interest shown in prenylamine, a coronary dilator whose spectrum of activity resembles reserpine. Prenylamine interferes with the incorporation of catecholamines into storage granules of the adrenal medulla and adrenergic nerves. Carlsson proposed that reserpine and prenylamine have common sites of action on monoamine storage granules.[65] Vieth studied prenylamine's effect on the CNS and concluded the drug was neuroleptic.[66] Prenylamine increases brain levels of monoamine metabolites, consistent with a depletion of brain serotonin and catecholamines.[67] Prenylamine decreases heart rate and arterial blood pressure in conscious animals but affects no change in anesthetized animals.[68] Obinawu found the pharmacology of prenylamine consistent with its amine-depleting activity and suggests an additional *in vitro* alpha and beta-adrenergic receptor blocking activity. Obinawu proposes that the beta-blocking activity predominates *in vivo*.[69] Szekers and Papp suggested that prenylamine has beta-stimulating activity since the increase in canine CBF is diminished by pretreatment with propranolol.[70] Nielson recently studied the depletion of catecholamines from the mouse heart and suggests that prenylamine depletes adrenaline stores within myocardial muscle, but does not affect the amine storage vessels in vascular tissue. The net result would be an interference with the metabolic and oxygen wasting effects of catecholamines while perserving their influence on vascular tone.[71] In man, prenylamine has mild sedative properties and diminishes the pressor response to tyramine.[72] In a double blind trial in angina pectoris, prenylamine was not significantly different from placebo in its effect on either subjective symptoms or objective changes in ECG or exercise tolerance.[73]

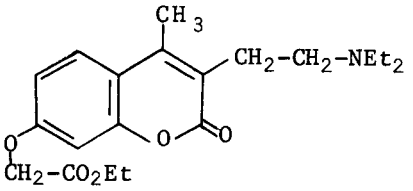
There have been a number of reports dealing with the actions of iproveratril (VIII). Iproveratril was claimed to be a beta-adrenergic receptor blocking agent on the basis of its antagonism to catecholamine-induced changes in blood pressure[74] and its bradycardia and antiarrhythmic properties.[75] Recent work has demonstrated that iproveratril is not a beta-adrenergic blocking agent.[76-79] Electromagnetic flowmeters were used to study iproveratril's effect on cardiac function and blood flow in the hind limb, kidney, and small intestine of cats. Iproveratril produced a hypotensive action by peripheral vasodilation, while large doses reduced cardiac output. All effective doses produced bradycardia. At high doses, iproveratril antagonized the chronotropic action of isoproterenol, but did not prevent isoproterenol-produced increases in stroke volume or aortic flow.[80] Haas compared iproveratril and a number of its analogues



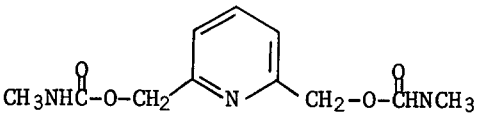
I Amiodarone



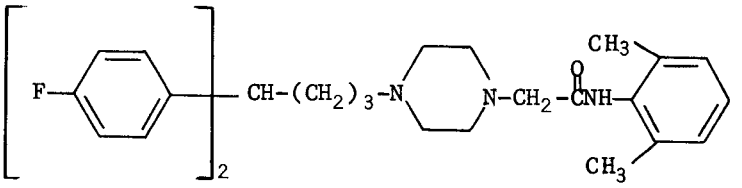
II Visnadine



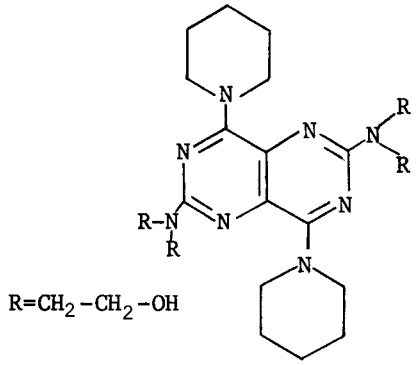
III Carbochromene



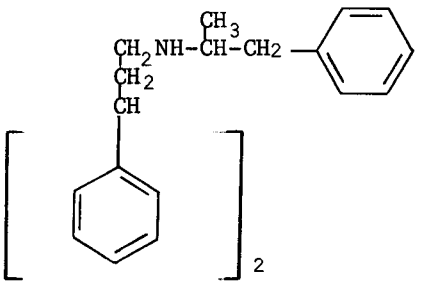
IV Pyridinolcarbamate



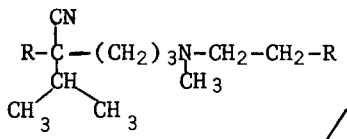
V Lidoflazine



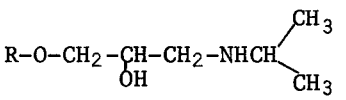
VI Dipyridamole



VII Prenylamine



VIII Iproveratril



IX Propranolol, R=1-naphthyl
X H56/28, R=o-allylphenyl

with propranolol in the rat. Iproveratril reduced the hypertension caused by stimulation of the rat central sympathetic nerve while known beta-blocking agents enhanced this effect. Iproveratril had no effect on the hyperglycemic response; it enhanced the hypokalemic effects and inhibited changes in free fatty acid caused by catecholamines. Known beta-blocking agents antagonized the hyperglycemic, hypokalemic, and plasma free fatty acid changes caused by catecholamines. Iproveratril was also observed to increase myocardial conduction time in dogs.[81] In guinea pigs, iproveratril is reported to prevent asphyxia-produced changes in ATP, creatine phosphate, AMP, orthophosphate, and ECG.[82]

In subjects without heart disease, iproveratril produced a 31% increase in CBF during the first 7 minutes, and no increase in the next 7 minutes. In anginal patients, iproveratril produced a 5% decrease in CBF during the first 14-minute interval.[54] During a cineangiographic study of 10 anginal patients, iproveratril produced a drop in systolic, diastolic, and mean and differential arterial blood pressures, accompanied by an increase in heart rate. No changes in the size of the main coronary vessels were observed, nor were there any changes in CBF as represented by a greater increase in the density of heart muscle during the capillary phase of coronary angiography.[83] A double blind study of iproveratril demonstrated a decrease in the daily consumption of nitroglycerin.[84] An uncontrolled evaluation of iproveratril in angina pectoris reports improvements in the subjective symptoms of angina.[82]

Dipyridamole (VI) produces coronary vasodilation by potentiating the effects of adenosine. Adenosine potentiation is believed to be a result of either direct adenosine deaminase inhibition or a result of alterations in red blood cell membranes which prevents adenosine uptake.[85-87] Dipyridamole enhances the vasodilator action of ATP. However, this effect is probably due to dipyridamole's action on adenosine breakdown, since ATP is dephosphorylated by whole blood.[88] Lozada reports that dipyridamole prevents heart mitochrondrial damage caused by hypoxia. Dipyridamole is believed to act on cellular membranes to maintain ATP levels within the mitochrondria.[89] Sordahl and Schwartz studied the respiration of mitochrondria from heart muscle and were unable to confirm previous reports that dipyridamole had beneficial effects on damaged mitochrondria. It was also postulated that dipyridamole inhibits mitochrondrial respiration by competing in electron transport systems. Dipyridamole is also capable of interacting with succinate-like substrates, producing an uncoupling of respiration.[90]

A number of studies have been conducted which confirm earlier reports that dipyridamole promotes the development of collateral vessels in animals with experimentally-produced myocardial infarction.[91-95] In man, dipyridamole decreases arterial levels of lactic acid, pyruvate, glucose, and free fatty acids.[96] A recent double blind study failed to substantiate earlier claims that any beneficial effects of dipyridamole would be related to the development of intercoronary collateral vessels, and that such changes would be observed only after long term treatment. There was no statistical difference in the effects of dipyridamole and placebo on

nitroglycerin consumption, ECG signs of ischemia, or the subjective symptoms of angina.[97]

A recent open study of itramine tosylate (2-aminoethylnitrate-p-toluenesulfonate) led to the conclusion that the drug favorably effected angina patients.[98]

Aerosol preparations of nitroglycerin have been promoted abroad for the relief of anginal attacks. Aerosol preparations were found to be no more effective than placebo in influencing exercise tolerance, incidence and duration anginal attacks or ischemic changes in the ECG.[99]

1967 marks the 100th anniversary of nitroglycerin's use in anginal therapy. Despite its shortcomings, nitroglycerin remains the standard by which other antianginal agents are judged. In his report of 1867, Brunton suggested that nitrates prevent the increase in blood pressure which ordinarily precipitates anginal attacks, and that relief of pain is achieved by reducing cardiac work.[100] Brunton's hypothesis was abandoned when it was shown that isolated coronary artery segments are relaxed by nitroglycerin and that nitroglycerin increases CBF in isolated heart preparations. With the failure of coronary vasodilators to improve the anginal syndrome, there has been a return to Brunton's earlier proposals.

The current status of nitrates has been recently reviewed.[101-103] There has been a great deal of interest shown in the biochemical effects of nitroglycerin. Needleman previously proposed that nitrates exert their therapeutic effect by reacting with SH groups within heart mitochondria, resulting in an uncoupling of phosphorylation.[104] Later work by Blum determined that this effect is related to the preparation method and substrate used. It was suggested that nitroglycerin restores the efficiency of aged mitochondria by uncoupling phosphorylation and by altering electron transport systems.[105] Recent work by Bing's group has shown that nitroglycerin inhibits monoamine oxidase (MAO) in vivo and in vitro. Since rather high concentrations of nitroglycerin are required for in vitro inhibition, Bing proposed that the in vivo effects might be a result of cellular concentration of nitroglycerin.[106] Inhibition of MAO could account for the observation that urine levels of vanilmandelic acid are decreased 12% following administration of nitroglycerin to humans.[107]

Beck suggested that differences in myocardial blood supply might produce localized cooling of heart muscle, which in turn could produce a congealing of protoplasm and cause stress on nerve endings. Experimental work failed to demonstrate any temperature difference in normal or ischemic tissue before or after nitroglycerin or myocardial revascularization.[108] Numerous studies have confirmed earlier observations that nitroglycerin may increase CBF in normal subjects, while CBF in anginal patients is either unchanged or decreased.[17,54,109-111] Sowton recently used atrial pacing techniques to study the hemodynamic effects of nitroglycerin during anginal attacks. Nitroglycerin reduced systemic pressure, heart size, cardiac output, stroke volume, and aortic pressure. The end diastolic heart size was reduced by nitroglycerin at all heart rates.[112]

Nitroglycerin's actions on the mechanical performance of the heart do not completely explain its mode of action. A comparison of nitroglycerin and dipyridamole's effect on myocardial resistance and flow suggests other mechanisms which may serve as a basis for the design of new antianginal agents. Nitroglycerin does not interfere with the autoregulation of CBF, and when administered to normal individuals, causes little change in coronary sinus or myocardial $P\text{-}O_2$. Dipyridamole, on the other hand, produces a marked elevation of coronary sinus and myocardial $P\text{-}O_2$ levels by upsetting the normal autoregulatory mechanism. Fam and McGregor proposed that nitroglycerin acts primarily on the large coronary arteries and conductive vessels without influencing the function of small arterioles and capillaries. In animals with chronic myocardial ischemia, nitroglycerin produces a rise in the $P\text{-}O_2$ of ischemic tissue, but does not change the coronary sinus $P\text{-}O_2$. Dipyridamole produces a fall in the $P\text{-}O_2$ of ischemic tissue and a rise in the $P\text{-}O_2$ of normal tissue and coronary sinus.[113] The fall in oxygen levels of ischemic tissue is a result of decreased vascular resistance in the normal myocardium, which diverts blood away from ischemic areas. These changes in oxygen content of normal and ischemic tissue are consistent with the effects of nitroglycerin and dipyridamole on retrograde flow.[114] These observations may offer an explanation for the uniform failure of coronary vasodilators in angina pectoris. Vessels in ischemic portions of the myocardium are already maximally dilated by an autoregulatory mechanism. Coronary vasodilators produce an upset of autoregulation in normal tissue, thereby diverting blood from ischemic areas. Increases in coronary sinus oxygen content and CBF are thus achieved by increasing flow through healthy areas of the myocardium at the expense of ischemic tissue. On this basis, coronary vasodilators would not only be ineffective in treating angina pectoris, but would present an actual threat to the well being of anginal patients.

The development of beta-adrenergic receptor blocking agents represents a significant departure from the classical design of antianginal agents. Beta-blocking agents appear to offer a rationale approach to anginal therapy.[115-117] These agents are not coronary vasodilators and may actually cause a constriction of coronary arteries and reduction in CBF. The effect of catecholamines and receptor-blocking agents on cardiovascular performance and CBF has been extensively studied.[118-123] The structure activity relationships of a large number of beta-blocking agents have been reported.[124]

Dwyer reports improvement in anginal patients treated with propranolol, (IX) which he ascribes to improvements in the exercise response to left ventricular pressure development and heart rate.[125] Parker found that propranolol produced an increase in left ventricular end diastolic pressure at rest and during exercise in the presence of decreased left ventricular stroke work. Parker suggests that these changes are a result of depression in myocardial contractility.[126] Grandjean compared a number of beta-blockers and found no correlation between the fall in cardiac output and the severity of heart disease, which suggests a negligible depression of contractility.[127] Bing's group used new experimental methods to study nitroglycerin and propranolol. In anginal and normal subjects, nitroglycerin

reduced shortening and velocity of shortening of ventricular fibers associated with a rise in myocardial oxidation-reduction potentials. Propranolol produced similar effects on ventricular fibers, accompanied by an increase in left ventricular end diastolic pressure and tension time index. In normal patients, propranolol had no effect on myocardial oxidation-reduction potentials. In anginal patients, there was a decrease in potential, indicating an impairment of myocardial oxygenation.[24]

Clinical trials of propranolol report reductions in the incidence of anginal attacks and nitroglycerin consumption and increased exercise tolerance. The amount of work necessary to produce ischemic ST depression is not generally increased. The most striking hemodynamic effects are decreases in resting and exercise heart rate and exercise blood pressure.[128-130] Gorlin's group recently reported that propranolol improved the short-term prognosis of anginal patients with documented coronary artery disease.[131] Improved prognosis with propranolol may be a result of reductions in cardiac energy expenditure and propranolol's anti-arrhythmic action.

Synergism of propranolol with various nitrates has been found. The use of long acting nitrates is believed to prevent propranolol-induced decreases in CBF and myocardial oxygenation. Improvements in pain, ischemic ECG patterns, and exercise tolerance are reported.[132-134]

Several new beta-adrenergic blocking agents have been reported which have a slight beta-stimulating activity. The intrinsic stimulation is claimed to be an advantage, as these compounds produce beta-blockade without depressing cardiac output or myocardial contractility.[127,135] Early trials of H56/28 report it to be beneficial in angina pectoris.[136]

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Chapter 9. Pulmonary and Antiallergy Agents

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The definitive characteristics of the three major types of obstructive lung disease (bronchitis, asthma and pulmonary emphysema) were previously reviewed in this chapter[1] and acknowledgment made of certain common features. These latter were recently elaborated to include factors possibly functioning etiologically in the progression from one disease state to another.[2] The merit and necessity of both the continued exploration of etiologic factors or conditions and the development of improved experimental laboratory models, as important prerequisites to rational and effective therapy of obstructive lung disease, was reflected in the past year by the considerable effort in these areas. A substantial impetus to much of this work was afforded by the development and increased utility of techniques permitting the simultaneous, quantitative assessment of pulmonary compliance and airway resistance in laboratory animals.[3]

Experimental Models and Etiologic Considerations. -- The hypersecretion and retention of bronchial mucus elicited in the rat by repeated daily inhalation of SO_2 was used for the evaluation of oral mucolytic drug action.[4] Such an *in vivo* technique has obvious importance as a confirmatory extension of the usual *in vitro* studies oriented toward physicochemical measurements. This method did not, however, afford any new insight into the transport or biosynthesis of mucus, and no other significant work in this potentially productive area was reported during the past year. Studies concerned with the pulmonary effects of inhalation of irritants other than SO_2 included NO_2 in rats[5] and cigarette smoke (cited[6] as the most important cause of broncho-pulmonary disease in the United States) in dogs[7] and other species.[8] Rats exposed to the atmospheric pollutant NO_2 showed peroxidation in lung lipids.[5] Lipoperoxidation and free radical formation have been implicated in the uncoupling of oxidative phosphorylation[9] and in the initiation of lysosome breakdown in the aging process.[10] These biochemical phenomena bear at least potential relevance to the progressive and irreversible destruction of alveolar and parenchymal tissue characteristic of emphysema in the older population.

Kuno and Staub[11] reported a technique for the mechanical production of microholes in excised, air-filled cat lungs and discussed the similarity of these fenestrations to those occurring as an early and fundamental defect in the development of pulmonary emphysema. A combination of tracheal constriction and the intra-tracheal injection of papain[12] or phytohemagglutinin[13] in immature rats induced a condition mimicing pulmonary emphysema as documented by histologic techniques and lung function measurements. Exposure of these emphysematous rats to cigarette smoke resulted in a reversible elevation in pulmonary resistance which was apparently mediated by changes in bronchomotor tonus.[14] These results suggest actual airway constriction as the intermediate mechanism subserving whatever

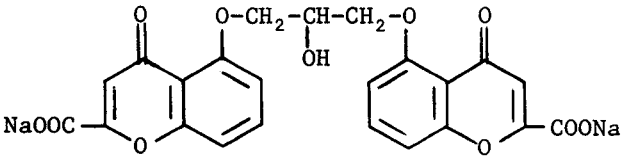
contribution cigarette smoking may make to the pathogenesis of emphysema. They are also perhaps analogous to the unrecognized airway obstruction reportedly associated with cigarette smoking by humans.[15] Repeated daily injections of progesterone prevented the development of emphysema in this experimental model.[13] These results are in agreement with clinical impressions of attenuation of emphysema during pregnancy and with the lower incidence of pulmonary emphysema in females than in males.

Biochemical studies[16,17] of human pulmonary connective tissue showed alterations in protein content in emphysematous lungs as compared to non-diseased tissue. Fibrous collagen was neither reduced in total amount nor altered in type. Enzymatic digestion of the elastic protein by pancreatic elastase likewise revealed no difference in large glycoproteins. However, the small sized polypeptide fraction showed marked increases in aromatic and basic amino acids, coupled with reduced content of neutral amino acids. The possibility is suggested[17] that a genetic disorder involving elastic protein synthesis or degradation may be operative in connective tissue during pulmonary emphysema.

Among the multiple processes involved in the pathogenesis of bronchial asthma, partial blockade of the β-adrenergic receptor has been suggested as an etiologic factor. Although this is not a new hypothesis, several publications were addressed to this concept, either directly or indirectly, during the past year. Supporting evidence was obtained in animal experiments in which pretreatment with β-adrenergic blocking agents intensified the anaphylactic response induced in mice and guinea pigs by histamine[18] or by sensitization.[19] Similarly, β-adrenergic blockade in hay fever patients increased the ventilatory response to ragweed exposure.[20] Cardiovascular and metabolic responses to β-receptor stimulant drugs obtained in asthmatic patients were also completely[21] or partially[22] confirmatory. The pronounced decrease in ventilatory function induced in asthmatic subjects by propranolol, per se,[23] augurs caution in the use of β-receptor blocking drugs in this disease entity.

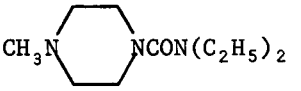
The prominent, but poorly understood, immunologic aspects concerned with the production and maintenance of bronchial asthma constitute the unique feature distinguishing this "respiratory allergy" from other obstructive lung diseases.[24] The symptom complex characterizing this respiratory allergic response is believed to be mediated by one or more chemical substances (histamine, acetylcholine, serotonin, bradykinin, and slow reacting substance) released or formed as a result of antigen-antibody union. This concept, assigning a basic etiologic function to the antigen-antibody union, provides the rationale for clinical trials with immunosuppressive agents in asthma. Because thiopurines interfere with antibody production,[25] 6-mercaptopurine and azathiopurine were studied for effectiveness in asthmatic patients -- both agents were found to be inactive.[25,26] Both of these purines produced encouraging results in treating other diseases thought to result from abnormal immune responses.[27] A new anti-allergic compound, disodium cromoglycate, was reported to have no bronchodilator nor corticosteroid activity and to be devoid of antagonism toward

histamine or slow reacting substance of anaphylaxis.[28] This compound appears to inhibit specifically the anaphylactic process initiated by reaginic antibody-antigen reactions.[29] Conflicting results were obtained in asthmatic patients with this agent, both when used alone,[30] and in combination with isoproterenol.[28,31] Thus, the efficacy of this anti-allergic compound in the treatment of bronchial asthma remains to be established.



Disodium Cromoglycate

A study of diethylcarbamazine in intractable steroid-dependent asthmatics[32] indicated symptomatic relief in daily oral doses of 10 mg/kg. This dosage exceeds that suggested for the chemotherapy of filariasis. In view of the reported success in this one study, confirmation of these findings would appear worthwhile.



Diethylcarbamazine

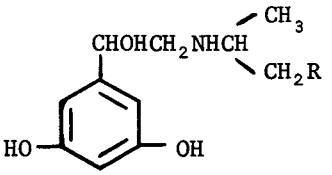
Two prostaglandins (PGF_{2a} and PGE_2) were reported to elicit opposite responses in human isolated bronchial muscle.[33] PGF_{2a} contracted, whereas PGE_2 relaxed, the isolated muscle. Both prostaglandins are present in human lungs[34] and the possibility of a functional relationship between the contractant PGF_{2a} and the relaxant PGE_2 was suggested.[33]

Bronchodilators. -- Epinephrine and isoproterenol serve as prototypes for sympathomimetic bronchodilators. The object of laboratory and clinical efforts in seeking new β-adrenergic stimulant drugs has been to obtain a bronchodilator which will be longer acting, have reduced cardiovascular side effects and be orally effective. In some cases of severe intrinsic asthma, it has been shown that the greater and more prolonged the broncho-constriction, the less the response to epinephrine-like drugs.

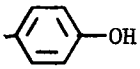
Isoetharine, although first reported in 1950,[35] has been the object of several recent clinical reports. Based on laboratory observations,[36] this alpha-ethyl homolog of isoproterenol preferentially retains broncho-dilator actions in relation to cardiovascular actions when compared to isoproterenol. In patients with obstructive lung disease, isoetharine was reported to show a long duration of action and selectivity for

bronchiolar musculature.[37] In these reports, isoetharine was combined with phenylephrine, an alpha adrenergic agonist, and thenyldiamine, an antihistamine.

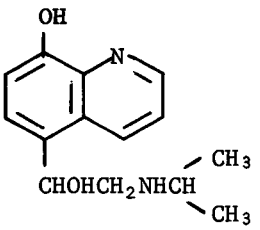
Metaproterenol, the 3,5-dihydroxy positional isomer of isoproterenol, continues to be reported as longer acting and more selective than isoproterenol in patients with obstructive lung disease.[38] A study of metaproterenol in combination with a tranquilizer, 7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2H-1,4-benzodiazapine-2-one, was performed. Using oral dosage and in ambulatory asthmatics, it was concluded that the combination was preferred to metaproterenol alone.[39] A single study of aerosolized metaproterenol in patients pretreated orally with either of the β-adrenergic blocking agents KO 592 or propranolol suggested a preferential retention of relaxant action on the lung relative to action on the heart.[40] This implies a specificity for β-adrenergic blockade of cardiac smooth muscle in relation to bronchial smooth and/or the reverse specificity for metaproterenol. This contrasts with other observations suggesting β-adrenergic blocking agents are contraindicated when β-adrenergic stimulant bronchodilator drugs are being employed in chronic bronchitis or asthma.[23] Th 1165a, an analogue of metaproterenol, was investigated in asthmatics and considered to be of superior potency.[41]



R = H = metaproterenol

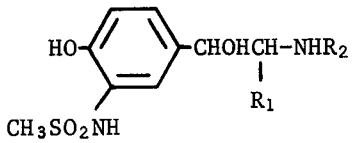
R =  = Th 1165a

A new isopropylaminoethanol derivative was reported this year.[42] This compound, quinprenaline, displays the 8-hydroxyquinoline chelating system and is the third such system investigated in the adrenergic field. The other two are the classical catechol system as in nor-epinephrine, epinephrine, etc., and the mixed catechol system involving the phenolic hydroxyl group and an alkanesulfonamido group.[43] Quinprenaline demonstrates many of the classic β-adrenergic stimulant effects, as characterized by isoproterenol, and is reported as quantitatively equiactive to isoproterenol orally or by inhalation versus histamine challenge. Its β-adrenergic stimulant actions were blocked by both sotalol and propanolol, β-adrenergic blocking drugs.



Quinprenaline

A large series of phenethanolamines bearing, on the benzene ring, both the alkanesulfonamido group and a phenolic hydroxyl group were recently reported.[43] Among the compounds discussed are β-adrenergic stimulant drugs with potent bronchodilator properties. Selected compounds of this series, as compared to isoproterenol, demonstrate a specificity of action for bronchiolar tissue in relation to cardiac or vascular tissue.



		Relative Potency	(Isoproterenol=1)
R_1	R_2	Tracheal Spiral	Depressor
H	$CH(CH_3)_2$	1.0	0.2
C_2H_5	$CH(CH_3)_2$	0.7	0.01
H	$CH_2CH_2C_6H_5$	0.3	0.02
C_2H_5	"	0.2	0.0005
H	$CH_2CH_2C_6H_5OCH_3$	0.6	0.01
C_2H_5	"	3.0	0.01

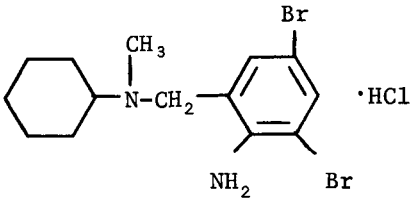
In the progression from hydrogen, to methyl, to ethyl on the α-carbon of the phenethanolamine there is a preferential retention of bronchodilator action, in relation to other actions, in this series just as reported for related compounds in the catechol series.[36]

Theophylline and its salts continue to find utility in the relief of airway obstruction. Recent studies indicate the observed bronchodilator effect of the xanthine,[44,45] as well as certain sympathomimetic bronchodilator agents,[46] may occur without a concomitant improvement in pulmonary arterial oxygenation. Increased concentration of inspired oxygen was suggested[44] as adjunctive therapy with the use of aminophylline. A clinical study[47] of aminophylline, β-hydroxypropyl theophylline and dihydropropyl theophylline in chronic bronchial asthma showed the first two compounds to evidence symptomatic relief, while the last compound and placebo had no effect on pulmonary ventilation. A laboratory study[48] of 7-(para-dimethylaminobenzo)theophylline (LJ 278) compared it favorably with theophylline itself. In this report, LJ·278 showed good oral absorption, no CNS stimulation and a marked coronary dilator effect accompanied by increased oxygen tension in coronary blood.

Mucolytics. -- Studies with acetylcysteine continue to show this compound, alone and in combination with isoproterenol, demonstrates a significant and real mucolytic action greater than that produced by water, saline, detergents or enzymes.[49] The principal mucolytic action of a sulfhydryl interchange with disulfide bonds in the mucoproteins appears to be

supplemented by a non-specific salt effect on other polymeric molecules such as DNA. Due to limitations of administration, acetylcysteine, or any other topically administered mucolytic agent, demonstrates greater effectiveness in the upper respiratory tract than in the lower, peripheral lung areas.[50] Several studies have shown that aerosolized 10% or 20% acetylcysteine can induce a bronchospasm in both asthmatic and nonasthmatic chronic obstructive lung disease patients. Concomitant administration of isoproterenol can eliminate or reduce this side effect.[51] A detailed laboratory study[52] of acetylcysteine showed rapid deacetylation to cysteine followed by classic cysteine catabolism. Inorganic sulfate was the main urinary excretory product. Oral administration of cysteine or acetylcysteine to rats or dogs produced little change in blood or tissue sulfhydryl levels and only a small increase in urinary sulfhydryl compounds. Acetylcysteine (10%) by inhalation was reported to have an effect no more deleterious than isotonic saline on pulmonary surfactant.[53] A review of pulmonary surfactant was published this past year.[54]

Bisolvon®, N-cyclohexyl-N-methyl-(2-amino-3,5-dibromobenzyl)-ammonium chloride, appears to possess several different modes of action which could make it useful in obstructive lung disease. The earlier studies reported from Europe have indicated utility by either the oral or topical (inhalation) route. Bisolvon® fragments the mucopolysaccharide fibers in sputum with a resultant decrease in viscosity. In chronic bronchitis there is reported an increase in sputum volume. At an optimal dose of 32-40 mg/day, in divided doses, increased pulmonary compliance, decreased respiratory work and improved alveolar gas tensions have been reported.[55] The actions of Bisolvon® on the bronchial epithelium of the rat have been characterized as increasing both the quality and quantity of secretions, with the suggestion that ciliate cells are converted into secreting cells.[56] On the basis of these reports, Bisolvon® is at least both an expectorant and a mucolytic agent.



Bisolvon®

An in vitro study of urea[57] indicated that an amount of urea necessary to exert a mucolytic effect in the respiratory tract could not be delivered by currently available methods of nebulization.

Respiratory Stimulants. -- Continued effort toward the synthesis of compounds as respiratory stimulants, and their examination in the laboratory and the clinic, was reported this past year. Some of this work was directed toward alleviation of respiratory depression induced by analgesic and anesthetic medication, where the depression is not intrinsi-

cally a respiratory or pulmonary problem.[58] There remain clinical situations where respiratory stimulation, in both amplitude and rate, is desired. In such instances there is generally an underlay of impaired gas exchange, and acute respiratory stimulation, together with other appropriate chronic therapy, may be beneficial. The specificity of action of such stimulants, i.e., respiratory center versus general CNS stimulation, does not appear to be large and it is difficult to assess how much the respiratory changes are due to a general alerting effect and how much to a specific stimulation of the respiratory center. Typical analeptics used in these situations are nikethamide,[59] ethamivan,[60] and a mixture of cropropamide and crotoethamide.[61]

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Section III - Chemotherapeutic Agents

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Bristol Laboratories, Syracuse, New York

Chapter 10. Antibiotics and Related Compounds

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General - Among the meritorious publications of 1967 concerning antibiotics of medicinal interest, several comprehensive works are particularly important. The four volumes of collected papers[1] presented at the Fifth International Congress of Chemotherapy in Vienna afford a perspective of current problems and progress. The impressive two-volume book edited by Gottlieb and Shaw[2] is a collaboration of many authors focusing on the mechanism of action and the biosynthesis of the best known antibiotics. A Polish book on more than 1000 antibiotic substances was translated into English.[3] An index to all antibiotics from actinomycetes up to 1966 appeared.[4]

Review articles of lesser scope include those on macrolide antibiotics,[5] water-soluble basic antibiotics,[6] polypeptide antibiotics,[7] and antibiotic classifications based on their mode of selective metabolic interference.[8,9,10]

This report is intended to provide key references to some of the most noteworthy chemotherapeutic advances in 1967, excluding the antiviral, antiparasitic, antifungal and antineoplastic antibiotics treated in subsequent chapters.

Infectious Drug Resistance - The world-wide increase in drug-resistant gram-negative *Enterobacteriacea* can be attributed to infectious drug resistance[11] wherein resistance is transmitted on extrachromosomal DNA (R factor) through conjugation. Transmissible resistance to eight different antimicrobial drugs was found in a strain of *Escherichia coli* isolated from clinical material. Resistance to all 8 drugs could be transferred to Salmonella and Shigella recipients within 2 minutes.[12] Five of 17 strains of *E. coli* (clinical isolates) were reported capable of transferring to *S. typhosa* their resistance to ampicillin, tetracycline, chloramphenicol, streptomycin and sulfanilamide.[13]

A multiple resistant donor strain does not necessarily transmit all of its resistance determinants to a recipient bacterium.[14]

Precise mechanisms of infectious resistance are coming to light. Two β-lactamases (penicillinases) were detected showing remarkably different substrate profiles.[13] A strain of streptomycin-resistant E. coli carrying R factor effected the inactivation via adenylylation of streptomycin.[15] Phosphorylative inactivation of kanamycin by Pseudomonas aeruginosa was reported.[16]

A combination of acriflavine and phenethyl alcohol was reported to "cure" R factor resistance to penicillin and chloramphenicol in a strain of Shigella flexneri.[17] The genetic material of R factors is most likely DNA; acridine derivatives are known to inhibit the replication of DNA.[18]

β-LACTAM ANTIBIOTICS

Allergenicity - Although there has been only 1 fatality attributed to penicillin per 100,000 patients treated[19] the chief contraindication to the use of the practically nontoxic β-lactam antibiotics is potential allergenicity.

A series of reports[20-22] presented evidence that a non-dialyzable retentate (ca. 0.02% by weight) from some commercial benzylpenicillin was more allergenic than protein-free benzylpenicillin ("Purapen G"). Comparative skin tests in two benzylpenicillin-hypersensitive individuals indicated a significantly reduced allergenic potential for the purified product. Whether there are therapeutic advantages to the use of deproteinized β-lactam antibiotics is under clinical investigation.[23,24]

Immune cross-reactivity of benzylpenicillin and cephalothin was reported.[25] Cross-allergenicity to penicillins and cephalorsporins was noted.[26] In contrast, in a study on the allergenicity and toxicity of cephaloridine and cephalothin, no cross-allergy with the penicillins was observed.[27] An orally effective cephalosporin, e.g. cephalexin, as well as oral penicillins, would appear to have greatly less sensitizing potential than those given by injection.

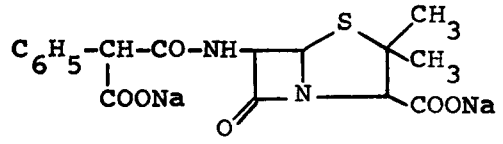
Methods for the detection of anti-penicilloyl antibodies by fluorescence polarization[28] and by penicilloylated bacteriophage[29] were described.

β-Lactamases and Amidases - Three types of β-lactamases were

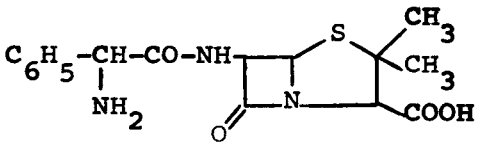
isolated from Bacillus cereus strain 569/H.[30,31] At least 8 "varieties" of β-lactamases produced by 4 different bacterial species have been examined[32] which differ markedly in substrate specificity, thermostability and susceptibility to conformational distortion by various penicillins and cephalosporins. β-Lactamases from gram-negative bacteria are intracellular and have "substrate-accessibility barriers."

The use of microbial amidases (acylases) for the production of 6-aminopenicillanic acid was reviewed.[33] An amidase from E. coli showed comparable efficacy in deacylating penicillins and cephalosporins containing the sterically similar phenylacetyl and thienylacetyl components.[34]

Broad-spectrum Penicillins - A signal advance in chemotherapy came with the discovery[35-37] that semisynthetic carbenicillin (disodium α-carboxybenzylpenicillin) (I) was clinically efficacious in the treatment of severe systemic infections of Pseudomonas aeruginosa (pyocyanea) and Proteus strains resistant to ampicillin (D-α-aminobenzylpenicillin) (II).



I



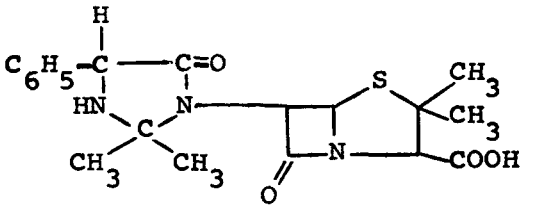
II

Carbenicillin has a broader range of antimicrobial activity than any other known penicillin. It is not absorbed orally and must be given by injection. Carbenicillin is synergistic with gentamicin, colistin and streptomycin.[38]

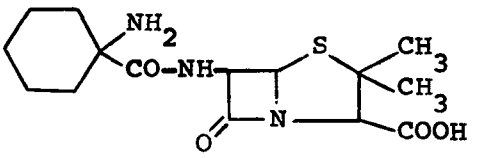
Prolonged ampicillin serum levels compared to those of benzylpenicillin were ascribed to the combined effects of resistance to degradation by the liver and to decreased renal clearance.[39] In the treatment of acute otitis media a single oral dose of ampicillin gave results at least equal to those obtained by combined drug therapy.[40] A combination of ampicillin and cloxacillin gave favorable results in the majority of patients treated for chronic bacteriuria.[41] The serum levels obtained with hetacillin (III) in man were compared with those given by ampicillin.[42]

Wy-4508 (1-aminocyclohexylpenicillin) (IV) is an example of broad-spectrum aminoalicyclic penicillins[43-46] which are efficiently absorbed orally and are more effective against

bacterial infections in mice than their *in vitro* activity would indicate. The *in vivo* activities of Wy-4508 and ampicillin were found to be comparable against typical gram-negative bacteria. Wy-4508 was as active as nafcillin *in vivo* against a penicillin-resistant strain of *Staphylococcus aureus*.

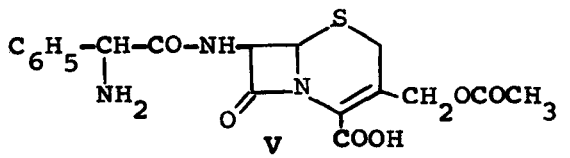


III

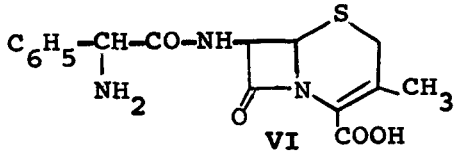


IV

Cephalosporins - Hydrogenolysis of the acetoxy group in cephaloglycin (V) produced cephalexin (VI), a broad-spectrum orally absorbed cephalosporin derivative.[47-49]



V

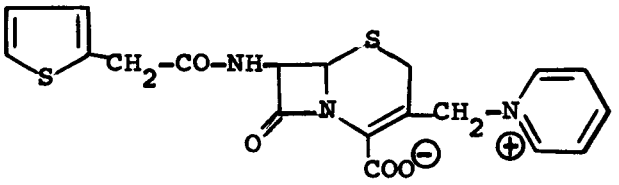


VI

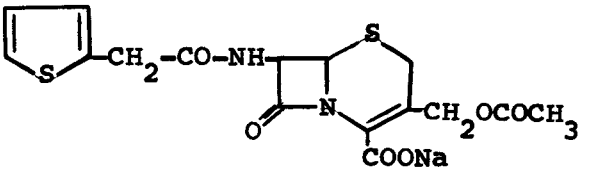
This transformation lowered *in vitro* activity but greatly enhanced stability and oral absorbability. Cephalexin is acid resistant, it is less than 20% serum bound, and it is almost quantitatively absorbed. About 96% of the orally ingested dose is excreted unchanged in the urine. No clinical side effects were noted.

Only 30% of orally administered cephaloglycin is excreted in the urine within 8 hours, at which time serum levels are negligible.[50] Cephaloglycin gave generally favorable results in combating urinary tract infections.[51-54] Cephaloglycin was very effective in treating streptococcal pharyngitis.[55] The general side effect noted in the trials was diarrhea.

Proceedings of a conference on cephaloridine (VII)[56] held at Oxford involved 55 papers and 148 participants. Some comparisons with cephalothin (VIII) are as follows:[57]



VII



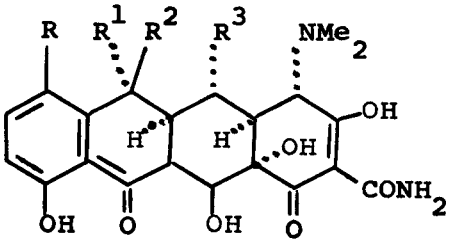
VIII

Property	VII	VIII
Serum protein binding	22.7%	61.8%
Serum half-life	90 min.	40 min.
Renal excretion (1 g I.M., 24 hrs.)	83.5%	75.6%

The metabolic fate of radioactive cephalothin and cephaloridine was determined in the rat.[58] A greatly improved synthesis of cephaloridine and analogs was reported.[59] Two excellent reviews on the cephalosporins were published.[60,61]

OTHER ANTIBIOTICS

Tetracyclines - Methacycline (IX) proved efficacious in 46 of 50 patients treated for respiratory infections.[62] No side effects were noted.



IX R = H, R^1R^2 = CH_2, R^3 = OH

X R = H, R^1 = CH_3, R^2 = H, R^3 = OH

XI R = NMe_2, R^1 = R^2 = R^3 = H

XII R = R^1 = R^2 = R^3 = H

Doxycycline (X), a hydrogenation product of methacycline, was marketed in the U.S. in September after a series of favorable clinical tests[63-66] disclosed high potency and efficient oral absorption for this more lipophilic chemical modification of oxytetracycline.

Metabolic studies using ^{14}C-labeled minocycline (XI) in the rat and dog showed a uniquely higher concentration of minocycline or metabolite in brain, thyroid and fat tissue than that obtained for other tetracyclines.[67]

Methods devised for the brilliant total synthesis of the microbiologically active dl-6-demethyl-6-deoxytetracycline (XII)[68] may well have opened the way to the total synthesis of novel tetracycline relatives not obtainable either by fermentation or by partial synthesis. Not unexpectedly, the in vitro antimicrobial activity of racemic XII was only half that of the natural isomer.

Chloramphenicol Analogs - From studies on the biosynthesis of chloramphenicol using ^{14}C-labeled compounds, it was concluded that p-aminophenylalanine is a specific precursor. Oxidation of the amino function gives rise to the nitro group in chloramphenicol.[69]

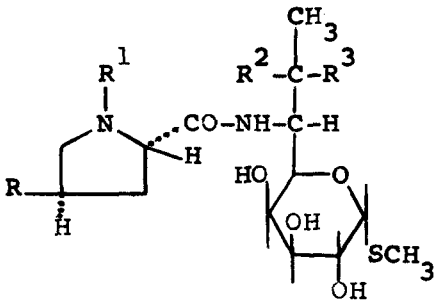
Cetophenicol, an analog of chloramphenicol having an acetyl group in place of the p-nitro group in chloramphenicol, was selected for clinical evaluation as an antimicrobial agent.[70]

An attempt to correlate the antimicrobial activity of chloramphenicol analogs with electronic polarizability led to the idea that the high potency shown by chloramphenicol and its methylthio analog may be bestowed, in part, by intramolecular charge transfer.[71]

Erythromycin - From 4 to 10% of children hospitalized with acute respiratory tract infections are afflicted with Mycoplasma pneumoniae (primary atypical pneumonia). In the search for more effective ways to combat this infection erythromycin proved to be clearly the most active of 21 commonly used antibiotics against 5 strains of M. pneumoniae in vitro.[72]

Lincomycin Analogs - Extensive systematic molecular modification[73,74] of lincomycin (XIII) produced the 7-chloro compound (XIV) which was five times as active as lincomycin against S. lutea in vitro and nearly twice as potent in vivo

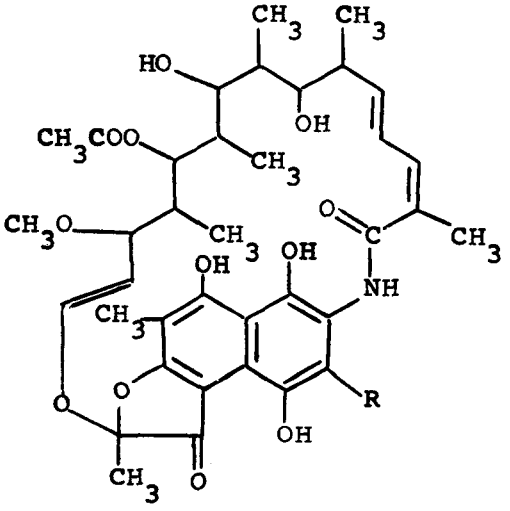
(mice, p.o.). Replacement of the 7-hydroxyl group by halogen significantly enhanced activity. Cis isomers were generally about one-half as active as the trans isomers. An N-ethyl (R^1) substituent tended to confer some activity against gram-negative bacteria, but not to an effective degree.



XIII R = n-C_3H_7, R^1 = CH_3, R^2 = OH, R^3 = H

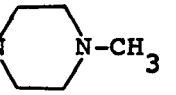
XIV R = n-C_5H_{11}, R^1 = CH_3, R^2 = H, R^3 = Cl

Rifamycins - From among the scores of semisynthetic rifamycins prepared from rifamycin SV (XV) and its 3-formyl derivative (XVI), Rifampicin (rifaldazine) (XVII)[75] stands out as a dramatic chemotherapeutic improvement over the parent compound.



XV R = H

XVI R = CHO

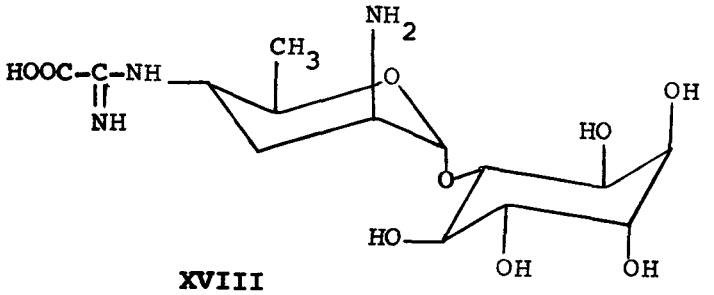
XVII R = CH=N- 

Rifampicin was found to be more active than cephaloridine, cephalothin, lincomycin and gentamicin against gram-positive bacteria. It showed no cross resistance with other antibiotics.[76] Rifampicin was as active as streptomycin

against M. tuberculosis in guinea pigs.[77] It was well absorbed orally by man.[78]

Clinically, Rifampicin gave unusually favorable results in the treatment of drug-resistant pulmonary tuberculosis.[79, 80]

Kasugamycin - An in vitro evaluation of kasugamycin (XVIII) led one investigator[81] to question the potential value of this aminoglycosidic antibiotic for the clinical treatment of pseudomonal infections. The results of a clinical trial, however, were interpreted as favorable. A dose of 0.5 g of kasugamycin given intramuscularly twice daily was reported to eradicate urinary tract infections without causing undue side effects, provided the patient had normal kidney function.[82]



XVIII

Tenemycin - A broad-spectrum antibiotic complex termed tenemycin is produced by Streptomyces tenebrarius. Four of the seven factors of the complex have been isolated and studied. They are aminoglycosides related to gentamicin, kanamycin and other 2-deoxystreptamine-containing antibiotics. Tenemycins are reported to be less toxic than related antibiotics and to be highly active against gram-negative pathogens, including strains of Pseudomonas.[83]

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Chapter 11. Synthetic Antibacterial Agents

Leonard Doub, Parke, Davis and Co., Ann Arbor, Mich.

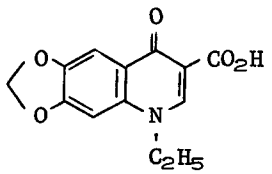
Introduction – No radically new antibacterial agent of proven worth appeared during this year. However, as has been true in each past year, many modifications have been made in existing types, a few of which show promise.

The material covered in this report is organized much in the pattern of previous years. Systemic agents have been first reviewed. Then attention has been turned to topical agents and to in vitro active compounds of unproven systemic potential. There are two kinds of exceptions to this pattern. A compound with only in vitro activity if closely related to a known class of systemic agent has been discussed with that class. For example, topical nitrofurans have been discussed with nitrofurans. Compounds with in vitro activity against the tubercle bacillus have been discussed with other antituberculous agents.

Reviews – Mayr's overview of chemotherapy has an excellent section on antibacterial chemotherapy.[1] During the year antituberculous agents from the clinical viewpoint were reviwed,[2,3] an assessment of isoniazid as a prophylactic was given,[4] and a clinical review of leprosy chemotherapy was given.[5] The background and clinical activity of the antileprosy phenazine compound B-663, was reviewed by Chang and discussed by him as a vindication of his murine leprosy test.[6] The status of leprosy prophylaxis with dapsone and with BCG vaccination was discussed.[7] The field of antiseptics and disinfectants was covered by Kretschmer.[8]

The Nalidixic Family of Antibacterials

Oxolinic Acid, I – Despite the exquisite specificity often shown to structural changes by the parent nalidixic acid II,[9] it is becoming apparent that some aspects of the molecule can be varied widely without loss of activity. Oxolinic Acid I is one such variant announced in 1967.[10] It has arrived at the stage of clinical evaluation.



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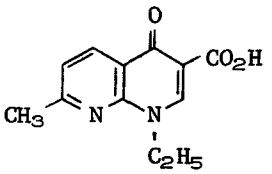
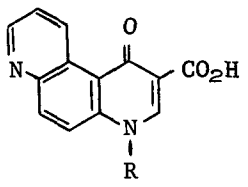
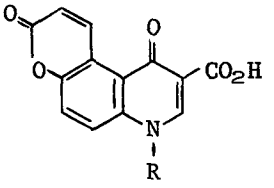
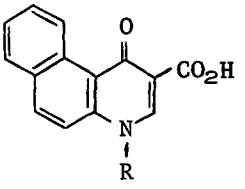


II

Like the parent, I is primarily active against gram negative bacteria and strains which become resistant are cross resistant to both drugs.[11] I is many-fold more active in vitro than II and 2-4 times more active in vivo.[12] I gives effective serum and urine levels in dogs and in mice.[13]

Early results of clinical trial in urinary tract infections suggest that I may be valuable in the control of E. coli and Proteus mirabilis but not other gram negative species (including other Proteus). Drug resistance was a prominent feature in cases failing to respond. Severe reactions were few, gastrointestinal upset was frequent.[14,15]

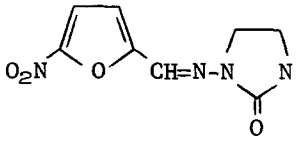
Polycyclic Analogs – Substantial activity in vitro and in vivo is retained in the following variations of the nalidixic pattern (R = lower alkyl).[16,17,18]

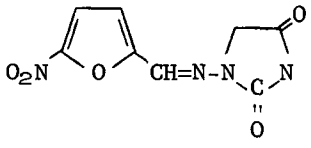
Biochemistry and Pharmacology – The effect of nalidixic acid on cell free systems suggests that the site of action for DNA in the intact cell is masked or absent in cell free systems.[19] Plasma and urine levels of I and its metabolites were determined in humans following a variety of dosage regimens.[20]

Nitrofurans

Oxafuradene III – Two preliminary clinical reports appeared this year comparing it to nitrofurantoin IV.[21,22] III is equally effective and as safe,



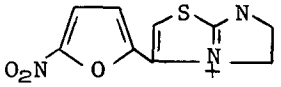
III



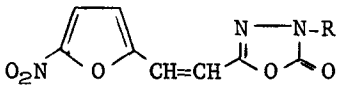
IV

but probably not superior to IV, despite its greater in vitro potency.

Furazolium Chloride V – Additional work was reported on this topical agent.



V



VI

Its 'cidal and 'static levels against a spectrum of gram negative and positive bacteria was determined. The drug applied locally can control fatally septic burns in rats.[23,24]

Nitrofuryl Vinyl Derivatives – As mentioned in previous reports, vinylogs

of active nitrofurans have received much attention; more papers appeared this year. The antibacterial activity and metabolic fate in humans was determined for VI (R = H or acetyl).[25]

Less than 1% of the acetyl compound was recovered in the urine (as activity). Effective serum levels were maintained for several hours. A large number of nitrofurylvinyl heterocycles were prepared and tested in vitro against a variety of bacteria.[26,27] The more active examples were substituted thiadiazoles, benzimidazoles and phenylpyrazolones.

Miscellaneous Nitrofuryl Derivatives and Allied Compounds — With the continued acceptance of nitrofurans as antibacterials and with the success of nitrothiophenes, nitrothiazoles, and nitroimidazoles in allied fields of chemotherapy, derivatizing continues apace. In vitro testing was reported for (substituted) semicarbazones and (substituted) thiosemicarbazones of nitrofurfuraldehyde,[28] (ring) N substituted nitrofurantoin,[29] methylpyrazoles,[30] aryltetrazoles,[31] carbonyl substituted isoxazoles,[32] oxadiazolines (none active in vivo),[33] aminocyanoisoxazoles (some active in vivo),[34] and imidazolopyridine and other fused ring imidazole compounds.[35] Nitrothiophenealdehyde acetals, semicarbazones, and thiosemicarbazones were compared in vitro with analogous nitrofurfuraldehyde derivatives.[36] The polymeric hydrazone from nitrofurfuraldehyde and polyacryloylhydrazide was active in vitro but not absorbed perorally.[37]

Mode of Action and Pharmacology — This important group of compounds continues to be the subject of theoretical and basic research. During the year a mathematical (molecular orbital) treatment of nitrofuran activity was given; it was deduced that bacterial action depends heavily on the reduction of the nitro groups.[38] The effect of crystal size on the rate of peroral absorption of nitrofurantoin and the effect on its excretion into the urine and on emesis was studied in rats, dogs and man.[39] Furazolidone as an inhibitor of monoamine oxidase was studied.[40,41]

Sulfonamides

General — That sulfas continue resurgent in the post-antibiotic era is attested by the large number of papers dealing with these drugs. The majority published during the last year cover clinical pharmacological, biochemical and physical aspects of these drugs. However, a few papers describing new derivatives appeared.

New Derivatives — Prepared and tested in vitro only were the following sulfanilamidoheterocycles: chloromethyltrifluoromethyl, and methoxypyrimidines,[42,43] amino and chloro substituted pyrimidines,[44] phenylpyrazoles,[45,46] trimethylpyrazines,[47] and the glucuronide of sulfadimethoxine.[48]

Tested in vivo but devoid of interesting activity was a series of 5-sulfanilamido-as-triazines.[49] N^4-phthalylsulfamethoxypyridazine was tested extensively in mice and in men and recommended for treatment of dysentery and enterocolitis in cases where sulfa treatment is indicated.[50]

Meningococci and Carbonic Anhydrase Inhibition – Interest continues on the significance to therapy of the unique sensitivity of meningococci to carbonic anhydrase inhibitors (see previous Reports). Sanders reviewed this rationale and reported on the trial of the diuretic ethoxyzolamide in eight carriers of meninogococci.[51]

While this potent carbonic anhydrase inhibitor did not eradicate meningococci from any carrier, Sanders reported that it produced profound changes in the organisms shed during and after treatment. He recommended further clinical and epidemologic studies to find whether these changes diminish transmissibility or invasiveness.[51] In contrast, Vaichulis and Vedros conclude from their in vitro studies with sensitive strains and a recently isolated clinically resistant meningococcus that the mode of action of sulfas on Neisseria meningitidis is no different than on bacteria generally.[52]

Mode of Action and Structure-Activity Correlation – Patsch and Hoehne continuing earlier work found cross resistance between sulfas to be variable in many instances.[53,54] They tested a wide selection of N^1-sunstituted sulfanilamides against over 200 microorganisms which were originally clinical isolates. The usual clinical pathogens were well represented. Ranking sulfas by their bacteriostatic endpoints they found different rankings depending on the test organism. The greatest differences between sulfas were encountered with pseudomonas and staphlococcus species.

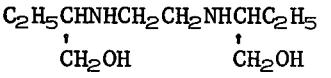
A challenge to a simplistic view is inherent in the above results. A sophisticated theory of action which can predict whether new structures have useful activity against selected pathogens is simply not at hand. However, this lack heightens interest in such broad, blunt structure-activity correlations as can be made. Value for activity-prediction of molecular orbital calculations,[55] and pKa and Hammet sigma values[56] was affirmed, while the usefulness of spectroscopically determined vibrational constants was disputed.[57,58]

Toxicity and Pharmacology – The long acting 4-sulfanilamido-5,6-dimethoxypyrimidine produced fewer side effects than, and gave equal therapy to, ampicillin in urinary tract infection.[59] Sulfasymazine (2-sulfanilamido-4,6-diethyl-s-triazine) was eliminated variably depending on diet, state of consciousness and a diurnal rhythm.[60] Sulfamethoxypyrazine and sulfamethoxypyridazine were compared for levels and persistence in the blood and organs of mice.[61] The renal and hepatic excretion of 20 sulfas was compared.[62] Terephthalic acid in the diet significantly increases the levels of sulfadimethoxine in rat and rabbit after single oral doses of the sulfa.[63] Acetylation and glucuronide formation was investigated for a number of sulfas in humans.[64,65,66,67] A toxic reaction new to sulfas was discovered with 3-sulfanilamido-6-ethoxypyridazine. On prolonged feeding at high doses it causes cataracts in dogs and rats.[68]

Antituberculous Drugs

Ethambutol – This drug was officially approved for marketing in the U.S.A.

last year.[69] This F.D.A. release comes some six years after start of test-

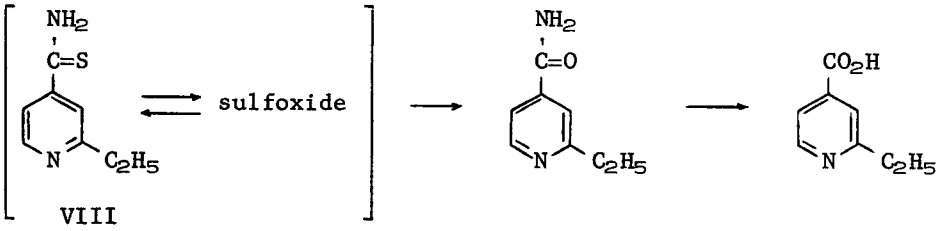


VII

ing. It is recommended for use as a companion drug with one of the standard drugs to prevent the emergence of resistant tubercle bacilli during treatment.[69,70] VII as a single drug treatment of tuberculosis is the subject of one paper.[71] Grassi has reviewed its experimental and clinical pharmacology, toxicology, and therapy.[72]

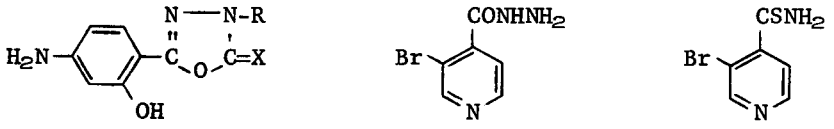
Thiocarbamyl Derivatives – Prepared and tested in vitro were numerous thiohydantoic acid derivatives, primarily hydrazides and hydrazones,[73] twenty-five aralkylthioureas and isonicotinoylthiosemicarbazides,[74] indoylalkylthioureas,[75] 1-(subst)aryl-3-heterocyclicthioureas,[76] mercaptoquinazolones,[77] and aryl (or) hetero vinylthioamides (i.e. vinylogs).[78]

Ethionamide, VIII, received some attention; it is metabolized in rats and mice as follows,[79] along with other metabolites. Seydel studied the



hydrolysis of VIII under physiological conditions (pH 7, 37°).[80] He found that the sulfur is readily eliminated and postulated that the toxicity of antituberculous activity is the pyridine ring and that the thioamide serves to aid penetration of VIII into the bacterial cell.

p-Aminosalicylic Acid (PAS) Derivatives – The substituted hydrazides of PAS were treated with phosgene or thiophosgene to give oxadiazoles (IX, R =



IX X XI

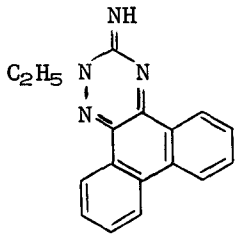
alkyl or H, X = O or S).[81] High tuberculostatic activity was found in both series; unfortunately no in vivo data was given.

Kakemi and coworkers determined that the in vitro action of omega substituted alkyl esters of PAS were reversed by added PABA and concluded

that the mode of action of these derivatives was similar to that of PAS.[81] They studied the absorption, biotransformation, and excretion and suggested that these PAS esters may have good sustained release qualities.[82]

Isoniazid (INH) and Derivatives – Various haloisonicotinic acid derivatives were prepared and tested in vitro by Palat and coworkers.[83] X was most active against the human strain $H_{37}Rv$, XI was most active against an INH resistant avian strain. Bekierkunst determined that INH affects nicotinamide metabolism and protects mice treated with cord factor (a toxic extract of tubercle bacillus). He discussed these results and proposed a mode of action of INH in tuberculous infections.[84]

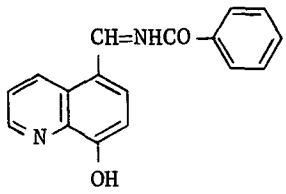
Miscellaneous New Antituberculous Compounds – The phenanthrotriazine (T.283, Hoechst) (XII) was active in mice infected with the atypical Mycobacterium kansasii.[85] It was somewhat less active than 4,4'-bis-iso-



·HCl

T.283

XII



XIII

amyloxythiocarbanilide. Tested in vitro were a series of phosphorylated aliphatic hydrazides,[86] p-phenylenediamines and their amides with isonicotinic acid,[87] and 2,4 and 5-formyl-8-quinolinols and their thiosemicarbazones and hydrazones.[88] The latter compounds were tested against both sensitive and resistant $H_{37}Rv$; with XIII there was cross resistance to INH.

Leprosy

New Outlook in Methodology – The extreme fastidiousness of the human leprosy bacillus has ever been a bar to meaningful test methods for agents to control human leprosy. In the past, reliance had to be placed for screening in the relevance of activity in related diseases; particularly relied upon in the past were activity in experimental tuberculosis and the almost equally unrelated murine leprosy. (See previous Reports). Since its inception about 1960, increasing use has been made of Shepard's method whereby human leprosy bacilli grow to a some extent in the footpads of mice. This year Rees and Waters reported a further advance.[89] They found that thymectomized and irradiated mice sustained an actively proliferating infection when inoculated with human leprosy bacilli. Their work has been hailed editorially as a model for lepromatous leprosy.[90,91] Already mentioned is Chang's feeling that murine leprosy has more relevance to the human disease than commonly believed, the history of B.663 being cited to support his thesis.[6] He and Vaituzis reported success this year in growing

M. lepramurium in mouse peritoneal macrophages,[92] possibly a model for an in vitro system.

Antibacterials with Limited or Unproved Systemic Potential

Developments in Established Topical Agents – The continued interest in nitrofurazolium chloride was covered in the section on nitrofurans. Substitution products related to dequalinium, (decamethylenequinaldinium salts), were described and their activities and toxicities given.[93] The metabolism in rats of Hg-labeled phenylmercuric acetate was studied.[94] Structure-activity reports were made for nitrotrifluoromethylanilides,[95] and salicylamides including replacement of the amide connecting group.[96] With high in vitro activity and synergism of nitrofurazone was 3,4-dichloro-4'-thiocyanatocarbanilide; its mouse toxicity was also given.[97] Williamson and Metcalf found several salicylanilides to be the most effective uncoupling agents for oxidative phosphorylation discovered so far.[98] Perhaps the mode of action of this important class of antiseptics has been found.

Miscellaneous In Vitro Actives – With antibacterial activity accompanied by some in vivo tolerance or nonbacterial chemotherapy data were the following additional reports: triazenoimidazole,[99,100,101] hydrazones of N-amino (cyclic) hexamethyleneimine,[102,103] further reports on antibacterial mesionic oxadiazoles,[104,105] oxamylhydroxamic acids,[106] 5-fluropyrimidines,[107] and a dinitropyrrole.[108]

Antibacterial activity, primarily in vitro, was reported for: azasteroids,[109] norethindrone,[110] indandionethiosemicarbazones,[111] phenylthiocyanates,[112,113] osazones from 3,4-dichlorophenylhydrazine,[114] tetramethyldipicrylamine,[115] fluoroanilinoquinaldines,[116] halogenated chalcones,[117] glycerylphenyl ethers,[118] benzofurans,[119] 2-mercaptobenzothiazoles,[120] aza analogs of isoleucine,[121] Mannich products from dichloropyridazinone,[122] pyrroles,[123] and N^1-adamantyl-4-aminobenzamide.[124] It is interesting that the action of the last compound is reversed by added p-aminobenzoic acid. The possibilities of N^1-adamantyl-4-aminobenzamide as a chemotherapeutic agent are under investigation.

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Chapter 12. Antiviral Agents

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During 1967 no new "antiviral" drugs were cleared for use in humans although activity was high at the laboratory and field trial level.

One comprehensive (1) and several more restricted reviews (2,3) were published. The former reviewed antiviral agents into 1966 with a strong orientation on the molecular biology approach. Great strides have been made in elucidating biochemical events peculiar to virus invasion of cells, but it is not yet possible to synthesize compounds with predictable useful "antiviral" activity on these findings. For the present a well-controlled screening program appears to be the most fruitful approach. Even here there are problems with considerable differences of opinion as to the definition of an "antiviral" agent and the best methods of finding such agents (4). Newer screening techniques described the use of the effect of antiviral compounds on the rales induced in mice by influenza virus (5), an automated screen using the inhibition of virus-induced nucleic acid synthesis as a parameter (6) as well as descriptions of the tight controls of all test parameters necessary to obtain reproducible results (7,8). Although a great deal of work on methods has been done, no single method has emerged as the best for discovery of antiviral drugs and there is still room for more effective techniques.

Interferon - Past reviews of this series excluded consideration of papers on interferon, but the rapid advances in this area merit their inclusion. Several recent reviews are available for background information on interferon (9,10,11). It is a protein or large polypeptide induced in cells by a number of agents including viruses which has the property of making cells refractory to virus multiplication (12) and is characterized by having a broad spectrum of antiviral activity but is specific for the species in which it is produced. It is produced by human cells and has been found in human fluids (13,14,15).

To date the antiviral activity of interferon has been demonstrated only in tissue culture or in animal systems and not in man. In one study (16) interferon induced by measles vaccine was found not to affect the shedding of cytomegalovirus of chronically infected children. However, this was not unexpected since this virus is highly resistant to the effect of interferon in tissue culture.

Two approaches for the application of the "interferon mechanism" to control of virus diseases have been considered - the direct use of

interferon produced exogenously and the induction of interferon in the host animal. Although direct application of interferon has provided protection of mice from Semliki Forest virus (17), the cell specificity and problems of making sufficient quantities of interferon for application render this approach unattractive (11).

The second approach, therefore, has been to discover non-viral inducers of interferon. Among the earliest virus inhibitors was helenine obtained from a mold, Penicillium funiculosum (18) which was shown to cause the formation of a viral inhibitor in mice with properties resembling interferon (19). Another such product, originally thought to be an anionic polysaccharide, is statolon produced by Penicillium stoloniferum and also found to be capable of inducing interferon as well as providing protection in virus infections of mice (20,21). Recently, following careful fractionation of the statolon preparations on sucrose gradients, it was found that active preparations contained numerous particles of typical virus morphology (22). All active preparations contained these particles and it was concluded that the interferon-inducing agent in statolon is not polysaccharide but a mold virus.

A brilliant series of experiments has shown that the active inducer from Penicillium funiculosum (helenine) is a double-stranded RNA which, when freed of inhibitory protein, is capable in microgram amounts of inducing interferon production in rabbits as well as protecting mice from virus infection (23). Subsequent studies indicated that microgram amounts of multistranded synthetic polynucleotide complexes as well as double-stranded RNA from reovirus and double-stranded RNA induced in E. coli cells by MS2 coliphage were also capable of inducing interferon production and host resistance in animals (24,25,26). Single-stranded RNA from a variety of sources as well as double-stranded DNA from calf thymus were inactive. The advantage of these inducers is the rapid appearance of interferon, two to four hours, compared with the much slower rise from virus infection. These materials can be produced in large scale, and although not yet tested in man, offer the potential for a broad-spectrum prophylactic antiviral agent.

A synthetic anionic polymer, first found to induce interferon in mice (27,28), has been tested for interferon induction in cancer patients (29). Fifteen to sixteen mg/kg of pyran copolymer (NSC-46015-C) was consistently effective in inducing interferon with a peak at 48 hours. The material is relatively toxic, inducing a high fever in most recipients and inducing thrombocytopenia at effective interferon-inducing concentrations. The effect of treatment against virus diseases in man was not tested and the material appears to be too toxic for general use although it should stimulate efforts to synthesize a safer material (30).

Other agents with reported activity in this area include phytohemagglutinin and pokeweed mitogen said to stimulate interferon production by human lymphocytes (31), Freund's adjuvant said to speed up interferon production in mice infected with foot-and-mouth disease virus (32), polyornithine (33) and egg polysaccharide (34) which apparently increase

uptake of exogenous interferon resulting in enhanced antiviral activity. Cortisol was found to suppress interferon production by E. coli endotoxin (a releaser) in rabbits but had no effect on interferon production induced by Newcastle Disease virus (35).

Although there are still problems such as cell refractoriness, interferon production inhibitors and lack of interferon response (36,37), it appears that initial antiviral clinical trials of interferon inducers could take place in 1968 (38).

Adamantanes - Of four reports analyzing the clinical and laboratory data on 1-adamantanamine HCl (amantadine HCl; "Symmetrel") through 1966, one (39) was highly critical of the significance of the findings whereas three (40,41,42) concluded that the data clearly supported the claims for prophylactic anti-influenza A2 activity in man. Continuing studies, both clinically and in the laboratory, have provided additional support of the antiviral activity of this drug. Data from a double-blind challenge study of A2 infection in man indicated that the total number of subjects with clinical and febrile illness was significantly reduced in the drug-treated group (43). One to 2.5 mg/kg/day of amantadine HCl administered to children in a double-blind prophylactic field trial was reported to significantly reduce respiratory illness (44). Part of the reduction was due to successful chemoprophylaxis of influenza A2 during an outbreak of this disease; the remainder was from a reduction of as yet etiologically unidentified upper respiratory illnesses. A report on studies carried out during minor influenza A2 epidemics in Sweden and the Netherlands during 1965 and 1966 also showed amantadine HCl to be clinically effective by prophylactic treatment (45). Concurrent therapeutic studies indicated a decrease in illness and some symptoms although this effect did not reach significance in all studies. Amantadine HCl was found to be inactive against parainfluenza type 1 virus in a challenge study in man (46), not unexpected since serum and nasal secretion levels of drug were lower than reported tissue culture effective concentrations. No serious side effects were reported in these studies in man.

A study in mice infected with influenza A2 indicated that aerosol administration of drug provided a high degree of protection, better than oral dosage (47). Antiviral activity was reported for amantadine HCl against Rous sarcoma in chickens and fowl leucosis in chick embryos (48), against fowl plague virus in tissue culture (49) and against multiple strains of influenza A/Equi-1 and A/Equi-2 in tissue culture, in ovo and in mice (50). Another study showed inhibition of the mitogenic response of human lymphocytes stimulated with phytohemagglutinin (51) and the suggestion was made that the drug might have a direct inhibitory effect on antibody response. However, it did not affect the antibody response induced in rabbits by either bovine serum or influenza A2/Japan/305/57 antigens (52) indicating that the photohemagglutinin result might be a special case of reaction with amantadine and cell membranes.

One analog, α-methyl-1-adamantanemethylamine HCl (EXP 126) has been tested in man by prophylactic treatment against a challenge infection with

influenza A2/Rockville/1/65 virus and shown to afford a high degree of protection against clinical disease (53). A number of adamantane derivatives were synthesized and tested in tissue culture and in mice infected with influenza virus. Several were antiviral, but amantadine HCl proved to have the highest degree of activity in both systems (54). These studies also suggested that the amino group was not essential for antiviral activity. Certain amines and ammonium salts were reported to inhibit influenza virus, especially A2 strains, in monkey kidney tissue culture (55). Like amantadine HCl, these worked at an early stage in virus reproduction. Other work on the mode of action of amantadine HCl supported the conclusion that the antiviral effect was at the stage of virus penetration and not on the stages of adsorption, replication or release (56). A tissue culture study of NH_4Cl on viral penetration indicated that this effect was too weak and variable to explain its antiviral activity (57). Unfortunately amantadine HCl was not tested since it and simpler amines have been reported to act by the same mechanism (58).

Thiosemicarbazones - Field studies on the use of 1-methylisatin-3-thiosemicarbazone (methisazone; "Marboran") were reviewed and the suggestion made that both treatment with compound and vaccination should be carried out on smallpox contacts for maximum protection (59). Studies on the use of methisazone in humans suffering from complications of smallpox vaccination indicated a high degree of efficacy against eczema vaccinatum, but a lesser degree of life-saving protection against the lethal complication of vaccinia gangrenosa (60,61). Although the viremia was apparently cured in a number of these cases, many eventually succumbed to an underlying disease. A summary article of the treatment of smallpox and complications of smallpox vaccination covers many aspects of the use of methisazone (62). In laboratory studies methisazone provided protection to mice infected IC or IN with smallpox virus when given prior to infection (63). In tissue culture methisazone inhibited varicella virus grown in primary human thyroid cells (64) and also showed activity against eight strains of adenovirus and SV15 grown in HeLa cells (65). Thirty μM provided complete inhibition and antiviral activity was present when compound was added 17 hours after infection. A number of substituted N-aminomethylisatins were synthesized and one, N-piperidinemethylisatin, was reported to have a high degree of activity against polio, type II; herpes simplex; measles and parainfluenza 3 (HA-1) although no test data were included (66).

Nucleosides - Although 5-iododeoxyuridine (idoxuridine; IUDR; "Stoxil"; "Herplex") has proven successful in the treatment of herpetic keratitis of the eyes, it has been less successful in the treatment of herpes simplex encephalitis. Using labelled IUDR it was found that insignificant amounts of IUDR entered the brain after IV injection (67). In addition, brain tissue was found to rapidly break down IUDR to IU $\rightarrow$ I + U and the authors concluded that this was the reason for lack of efficacy. Recent very limited studies in humans indicated that early treatment of herpes simplex encephalitis with IV IUDR and external decompression was not only life-saving, but prevented permanent brain damage

(68). In previous failures, diagnosis was delayed and treatment started after permanent damage occurred.

Preliminary studies with azauridine suggest that this material was effective for the treatment of herpes simplex and zoster infections of the human eye (69). Rabbits infected with vaccinia virus vaccine were completely protected from pustule formation and erythema by 2-3 grams of compound injected IV (70). This material apparently has a low order of toxicity. 5-Bromo-2-deoxyuridine was found to inhibit a new virus, equine herpes 3, in rabbit kidney cells as well as pseudorabies, a DNA virus, whereas vesicular stomatitis, an RNA virus, was resistant (71). A large number of nucleosides have been synthesized and tested in various systems for antiviral activity. 1-β-D-Arabinofuranosylcytosine (cytarabine; ara-C) blocked DNA synthesis of herpes-infected cells (72) while a number of structurally related compounds showed some antiviral activity, although less than the parent compound (73,74,75,76). It is still too early to determine the ultimate value of these newer agents as "antiviral" drugs.

Other Antiviral Substances - Ascorbic acid has been reputed to protect against and "abort" the common cold. This was not confirmed in tissue culture against a number of viruses including strains of rhinovirus and in influenza-infected mice in which no effect of ascorbic acid treatment on lung lesion score or mortality was observed (77). In spite of these negative laboratory results, the effect of 3 grams per day of ascorbic acid was studied in human volunteers challenged with a number of viruses causing upper respiratory disease. Again no protective or therapeutic activity was observed with the conclusion that ascorbic acid was valueless in the treatment of upper respiratory disease.

Viractin, for which there are unconfirmed claims of activity for the prevention of respiratory infection, once again was shown to be ineffective in a cross-over placebo-drug study in volunteers (78).

[N',N'-Anhydrobis(β-hydroxyethyl)biguanide HCl] (ABOB; "Virugon"), for which there are conflicting claims for activity against influenza virus, was studied in a population of 5744 industrial workers in Sweden. High levels, 1600 mg/day, given prophylactically reduced clinical influenza from 2.25% in the placebo group to 1.16% in the treated group (79). In the overall study there was no difference in other upper respiratory tract disease between placebo and drug groups nor was there any difference in side effects. ABOB treatment of adults and children resulted in a statistically significant reduction in the length of fever but not other symptoms of pharyngoconjunctival fever caused by adeno type 3 virus (80). Of other compounds tested in man, one analog, from a group of thiazolidineacetic acid derivatives with activity against herpes simplex in tissue culture, was reported to be active in the treatment of volunteers with labial or ocular herpes simplex infections (81).

Several natural products were reported to possess antiviral activity in laboratory animals. Extracts from narcissus and magnolia plants

provided protection against lymphocytic choriomeningitis infections of mice (82). Gymnenic acid extracted from Gymnema sylvestre was active against several viruses in tissue culture and also caused a small reduction in lung lesions of influenza-infected mice (83). A protein isolated from shellfish with weak activity against polio and influenza B in mice was found to be produced in the livers of the shellfish but not other tissues (84,85). A factor (phagicin) isolated from λ-phage-infected E. coli lysates active against DNA viruses was found to be a phage internal protein (86) and showed in vivo activity against herpetic keratitis in rabbits and cutaneous vaccinia infections of mice (87). Living or killed E. coli and a polysaccharide isolated from E. coli were found to inhibit herpes simplex in tissue culture whereas meningococcus was inactive (88). This was presumed to be through an interferon endotoxin mechanism.

Resulting from studies on the antivaccinia activity of pterygospermin, a number of substituted potassium benzylaminothiomethanesulphonates and related compounds were made of which several possessed antivaccinia and anti-influenza activity in tissue culture, in ovo and in animals (89,90,91). Part of the mode of action of these compounds appears to be due to the liberation of isothiocyanate.

The sodium salt of 1,1,3,3-tetracyanopropene (TCNP) was markedly antiviral by IP injection but inactive orally in mice against Columbia SK and herpes viruses with slight activity against coxsackievirus B1 (92). 1-(4-Fluorophenyl)-1-phenyl-2-propynyl-N-cyclohexyl carbamate (FPPC) caused a marked reduction of splenomegaly in mice infected with Friend leukemia virus as well as reducing tumor growth in chicks infected with Rous sarcoma virus (93). Caprochlorone [levo-4-phenyl-4-(2-chlorobenzyl)-5-oxy-hexanoic acid] showed activity against 15 myxoviruses and vaccinia with a mode of action similar to amantadine HCl (94). Reports on other antiviral agents in early stages of testing included dibenzylphosphinic acid with tissue culture activity against encephalomyocarditis virus (95), polyvinyl sulfate active against some strains of herpes simplex (96), a number of isoquinoline derivatives which had both virus inactivation and inhibition effects without cell toxicity (97), a number of 1,2,4-triazole derivatives (98), N-acyl derivatives of pyrimidine and purine bases (99) and N-acyl derivatives of 5-triazine bases (100), all with reported tissue culture activity against several strains of viruses. A compound found to selectively inhibit viral RNA polymerase was active in mice against influenza PR8 infections as well as blocking Rous sarcoma tumors in chicks (101). Most of these latter agents are in preliminary laboratory stages and much work remains before any can be considered as antiviral drugs.

Rapid Diagnosis - With the advent of antiviral drugs and their narrow spectrum of activity, it became apparent that rapid diagnostic methods were necessary for full use of these new agents. The classic virus identification systems are far too slow to be of value. One approach for the rapid diagnosis of a variety of viruses has been described (102). Another approach is that of fluorescent antibody (FA) which is rapid but highly specific (103,104). The FA technique is satisfactory

so long as there are not a large number of highly specific antiviral agents, each requiring a specific fluorescent antibody. This method has been used for the early diagnosis of herpetic encephalitis which was successfully treated with IUDR (68). It is hoped that rapid diagnostic measures will keep pace with progress on antiviral drugs.

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Chapter 13a. Human Antiparasitic Agents

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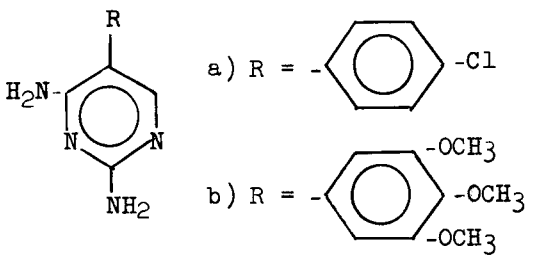
Introduction - Several reviews of parasitic diseases and their chemotherapy have been published.[1-7] Although some progress has been made during the past year in the treatment of resistant Plasmodium falciparum infections and in a better understanding of the action of drugs in schistosomiasis, the problems related to the prophylaxis and treatment of many parasitic diseases are not yet fully resolved. There is no adequate treatment and no new drugs have appeared on the horizon for Chagas' disease, toxoplasmosis, visceral larval migrans and pneumocystis pneumonia. The latter disease is gaining prominence with the increasing use of immunosupressive agents in medicine.[8]

PROTOZOAN INFECTIONS

Malaria - According to WHO reports, malaria eradication is today an accomplished fact for 619 million people. An additional 334 million population live in areas where transmission of the parasite is no longer a major problem. On the other side of the picture there are another 638 million who live in areas where transmission still continues.[9] The number of reported cases of malaria in the U.S. is increasing rapidly, mainly due to military returnees from Vietnam.[10] In 1966 there were 565 reports as compared to 147 reports in 1965.[11]

The 4-aminoquinoline resistant P. falciparum malaria is still an important problem in specific areas. To date no authenticated cases of this resistant malaria have been documented in Africa.[12] There is no single, all purpose drug suitable for both prevention and cure of malaria. Interest in this area is shown by the number of reviews and major publications that have appeared within the last year.[12-17]

Current Therapy - Although 4-aminoquinoline resistant P. falciparum infections continue to be reported, the overwhelming majority of malaria infections are amenable to chloroquine therapy. Effective treatment of 4-aminoquinoline resistant falciparum malaria with combinations of two or more antimalarials including chloroquine, colchicine, quinine, pyrimethamine (Ia) long-acting sulfonamides and diaminodiphenylsulfone (DDS) have been reported.[18-24] A single 1-gm. dose of sulphormethoxine (Fanasil®) (IIa) and 50 mg. pyrimethamine produced radical cures in 17 of 19 patients in Thailand.[18] According to Sheehy and Reba,[19] a pyrimethamine-quinine treatment for all P. falciparum infections acquired by U.S. troops in Vietnam showed a cure rate of 90-95%. Supplemental therapy with DDS in patients treated initially with both chloroquine and quinine was highly effective in reducing recrudescence rates of P. falciparum.



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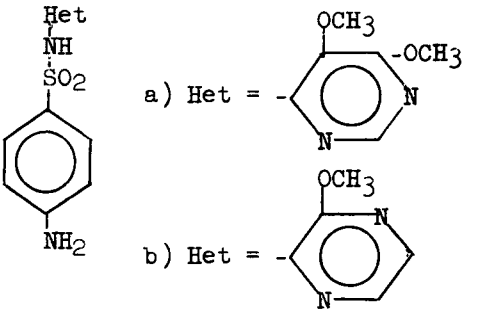

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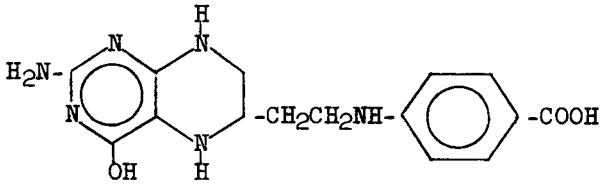
Modes of Action - A review of the mechanism of action of chloroquine is given by Sams.[25] The drug interferes with the action of several enzymes, binds to melanin and DNA, stabilizes lysosomes and blocks the sulfhydryldisulfide interchange reaction. In the presence of chloroquine-sensitive plasmodia (P. berghei), mouse erythrocytes concentrate chloroquine to levels more than twice as high as those parasitized by chloroquine-resistant plasmodia.[26] Further work on the structure of the chloroquine DNA complex indicates intercalation of chloroquine into DNA.[27] Support for the mode of action of quinacrine in impairing DNA replication in plasmodia is given by Ciak and Hahn.[28] Molecular orbital calculations of a number of representative antimalarial compounds was reported.[29] The experimentally observed interaction of chloroquine with the guanine of DNA seems to be explained satisfactorily by electron donating or accepting characteristics of the respective molecules.

Potentiation of antimalarial effects are obtained by combinations of drugs with varied mechanisms of action. The antimalarial effects of certain sulfones and sulfonamides apparently involve blockage of incorporation of p-aminobenzoic acid (PABA) in the formation of folic acid in the parasite. Pyrimethamine acts at a different point in the same metabolic pathway.[30] DDS may be competitive with PABA in the Zwitterion form.[31]

Potential Chemotherapeutic Agents - Field studies on the repository antimalarial agent, cycloguanil pamoate (CI-501) alone or in combination (CI-564) with 4,4'-diacetylaminodiphenylsulfone (CI-556) are continuing.[32-35] In a small number of cases CI-564 showed limited usefulness as a therapeutic agent.

Trimethoprim [2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine] (Ib) was reported to be effective against pyrimethamine and 4-aminoquinoline resistant P. falciparum infections in man.[36] In combination with 2-sulfanilimido-3-methoxypyrazine (IIb) a one-day treatment was promising.[37] Tetrahydrohomopteroic acid (THHP) (III) was reported to be the first of a new class of antimalarial antifolates and was found effective in pyrimethamine-resistant malaria in monkeys.[38,39] Plasmodium berghei is used widely to screen and evaluate antimalarials. A 7-halogenated lincomycin derivative (U 24,729A) (IV) was reported to be effective against a strain of P. berghei malaria in rodents resistant to chloroquine and DDS. Lincomycin

hydrochloride has no antimalarial activity of its own.[40] Prodigiosin (V) was also found to protect mice against P. berghei infections.[41]



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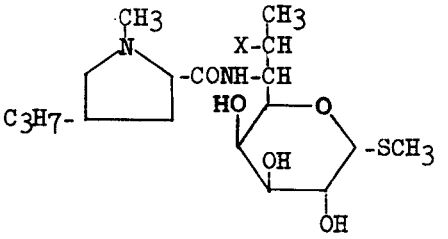
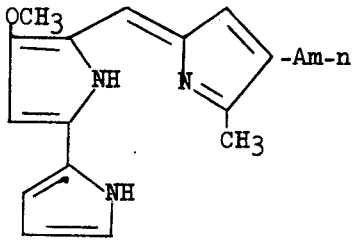


(IV)

There is renewed interest in the naphthoquinone antimalarials.[42,43] The 2-hydroxy-3-(ω-cyclohexylalkyl)-1,4-naphthoquinones (VI) with long alkyl chains are active.[42] Cyclization of derivatives of arylbiguanides and arylamidineureas with acetoacetic ester or acetylacetone to the corresponding pyrimidines (VII, Y = NH, O) reduced both the toxicity and antimalarial activity.[44] Compounds resulting from coupling sulfa drugs and pyrimidines showed antimalarial activity which appears to be due to the intact molecule.[45]



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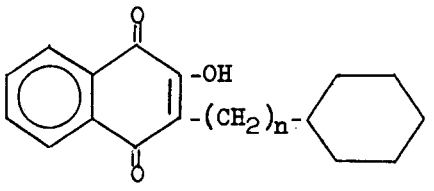

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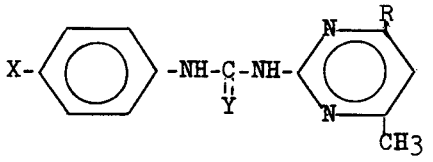
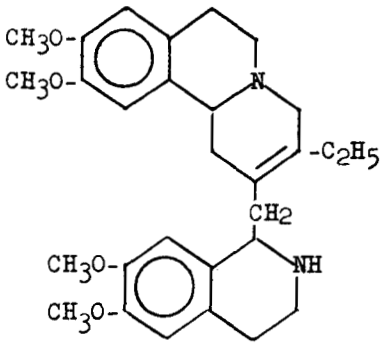


(VII)

Amebiasis - There have been no real advances made during the past year in the treatment of Entamoeba histolytica. A drug equally effective for the treatment of both systemic and intestinal amebiasis is not available.

Administered intramuscularly, dehydroemetine (VIII) is reported to be less cardiotoxic than emetine.[46] Recent reports have attested to the effectiveness of oral dehydroemetine in intestinal and extra-intestinal amebiasis.[47,48] Further support for the mechanism of action of emetine involving inhibition of protein synthesis is discussed by Grollman.[49] The advantages of chloroquine, which is still one of the drugs of choice in the treatment of systemic amebiasis, are its oral use and lack of cardiac toxicity. Other drugs which have been shown to be intestinal and systemic

amebacides are the nitrothiazole derivative, niridazole (Ambilhar®)[5] (IX), and the nitroimidazole compound, metronidazole (Flagyl®)[50] (X). The central nervous system toxicity of Ambilhar® restricts its use in amebiasis.[51,52]



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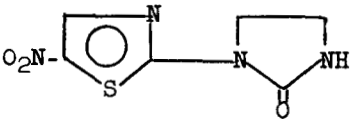

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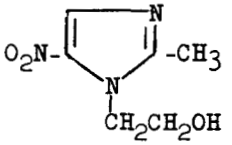
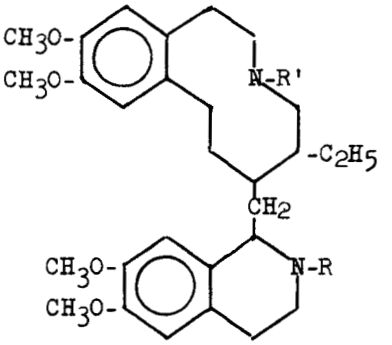
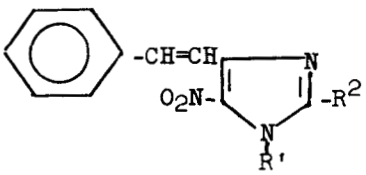


(X)

The preparation and the antiamebic activity of 1',2'-secoemetine (XI)[53] and styrylimidazole[54] derivatives (XII) have been reported. Diloxanide (XIIIa), one of the dichloroacetamide anti-amebic agents, appears to be dependent on the presence of viable bacteria for activity.[55]



(XI)



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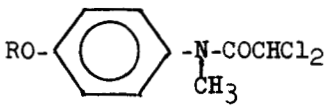
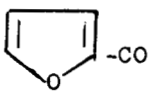


a) R = H b) R =

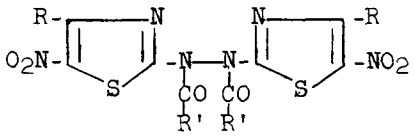


(XIII)

The results of clinical trials using combinations of anti-amebic drugs are encouraging. Powell[56] concluded that acute amebic dysentery should be treated with a combination of a direct acting, systemically effective amebacide (emetine preparations, quinoline derivatives) and a broad spectrum antibiotic (tetracyclines). In this connection, the

combination of diloxanide furoate (XIIIb), tetracycline and chloroquine has been used.[57,58]

Trichomoniasis - Oral metronidazole (X) continues to be the drug of choice in the treatment of trichomoniasis.[59] Some 1,2-diacyl-bis (5-nitro-2-thiazolyl) hydrazines (XIV) were found to be active in vitro against Trichomonas vaginalis, but were inactive against T. muris in mice.[60] A variety of 1,3,4-oxadiazolin-5-ones (XV) were found to be active in vitro against T. vaginalis as well as E. coli and Staph. aureus.[61] A large number of new 1-alkyl-5-nitroimidazoles (XVI) have been prepared and evaluated in mice for trichomonicidal activity (T. foetus). Some of these compounds were reported to be at least five times as potent as metronidazole. Variations in alkyl groups in positions 1, 2 and 4 affected the physical properties and biological activity of these compounds.[62]



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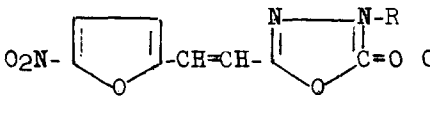
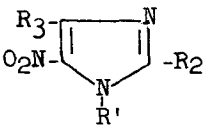


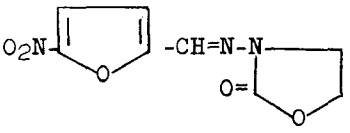
(XV)



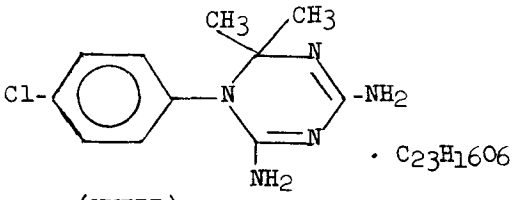
(XVI)

Giardiasis - Several recent publications have reported the efficacy of metronidazole in the treatment of Giardia lamblia infections. A daily dose of 20 mg/kg for 5-7 days cured 83% of 48 children.[63] An increase in the daily dose to 30 mg/kg for 7 days cured 27 of 30 children.[64] Of 17 patients (3-36 years) followed for 1-2 months after a 5-day course of treatment (270-500 mg/day), 16 were cured of the infection. The drug was reported to be well tolerated with few undesirable side effects.[65] In children, furazolidone (XVII), given for 4 or 5 days at 10 and 5 mg/kg/day respectively, produced cure rates of 75% to 87%.[66]

Leishmaniases - No new publications on the intramuscular antimalarial drug, cycloguanil pamoate (XVIII), for the treatment of cutaneous leishmaniasis[5] have been found in recent literature. Oral dehydroemetine (VIII) was reported useful in the treatment of leishmaniasis (L. tropica) known as oriental sore.[67,68] A 60% cure rate was obtained among 32 patients receiving the resinate or hydrochloride forms of the drug daily for 3 weeks to 3 months. Only minimal and transient side effects were seen.[67] In 30 cases of skin leishmaniasis due to L. mexicana, treatment with metronidazole (X) gave 78% recoveries. The oral dose was 250 mg. every 12 hours for 15 days.[69]



(XVII)



(XVIII)

METAZOAN INFECTIONS

Schistosomiases - The development of schistosomacides, more effective and with wider range of action than those presently available against the 3 principal human species, is still a great necessity for meeting the world problem of schistosomiases. The number of people who suffer from this disease has been estimated at 200 million and appears to be increasing. The debilitating consequences of the disease represent a tremendous economic loss.[70]

Several review articles on the laboratory and clinical evaluation of schistosomicidal substances have appeared during the past year.[51,71-76] A conference on niridazole and other antischistosomal compounds was held in New York, October 10-13, 1967.[77,78] A procedure for the removal of schistosome worms by surgery and extracorporeal filtration of portal blood has been developed for relief of severe hepato-splenic S. mansoni infections.[79,80]

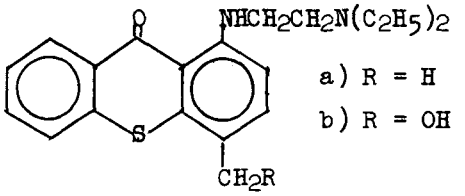
Current Therapy - Parenteral antimonials include antimony potassium tartrate (tartar emetic) or the corresponding sodium salt, Stibophen (Fuadin) [pentasodium antimony bis (catechol-3:5-disulfonate)] and potassium antimony, α,α'-dimercaptosuccinate (TWSb). Among the oral drugs now in use are lucanthone (Miracil D) (XIXa) and niridazole (IX). A human oral daily dose of 25 mg/kg of niridazole for 5-10 days is effective, particularly against S. haematobium.[81-84] The central nervous system toxicity observed in patients treated with this drug is reviewed by Weller and others.[51,77]

Modes of Action - Metabolic studies with niridazole indicate that it is broken down by an enzyme system having properties of a nitroreductase.[85] The inability of the liver in severe cases of schistosomiasis to effectively metabolize the compound may be associated with increased toxicity.[86,87] After a single dose to mice infected with S. mansoni, inactivation of phosphorylase by homogenates or extracts of the worms is reduced significantly.[88]

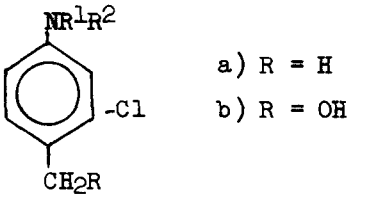
Lucanthone was shown to complex with DNA, thereby blocking cellular RNA synthesis and inhibiting bacterial growth (E. coli). The presence of spermine in the culture prevents this action.[89] 4-Iodoacetamidosalicylic acid (IAS) inactivated isolated S. mansoni lactate dehydrogenase, but has little or no effect on this enzyme from other sources including human serum. Although the compound is toxic to intact schistosomes in culture, there was no effect on the lactate dehydrogenase activity.[90]

Potential Chemotherapeutic Agents - The microbial transformation of lucanthone by Aspergillus sclerotiorum to give the hydroxymethyl compound, hycanthone (XIXb), the active metabolite, has been described.[91] Similar microbial metabolism of some N-substituted 3-chloro-p-toluidines (XXa) to the corresponding 4-hydroxymethyl derivatives (XXb) resulted in enhanced schistosomicidal activity in mice and hamsters.[92] Encouraging clinical

tolerance and efficacy of hycanthone given orally (2-3 mg/kg x 2/5 d.), was reported. Cure rates were 80-83%.[93]



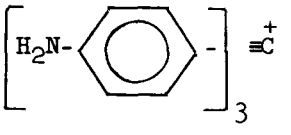
(XIX)



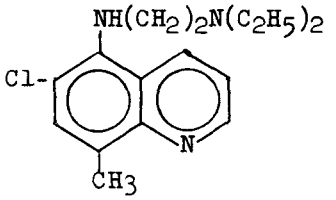
(XX)

The pamoate salt (CI 403-A) of tris (p-aminophenyl)carbonium (XXI) was effective in the treatment of S. japonicum when given orally in a maximum dosage of 35-40 mg/kg/day for as many as 52 days spread over a total treatment period of 203 days.[94] This is in accord with previous findings.[95] The effects of tris (p-aminophenyl)carbonium salts (TAC) on S. mansoni are as follows: 1) a reduction of glycogen in the cuticular tubercules of males, 2) morphologic and functional alterations on the female reproductive system that lead to abnormal egg production and 3) inhibition of acetylcholinesterase activity in the nervous system of the worm.[96]

The outstanding compound to show schistosomicidal activity in a series of 5-aminoquinolines was RD 12,869 [6-chloro-5-(2-diethylamino-ethylamino)-8-methylquinoline] (XXII).[97] Against S. mansoni in mice the compound was active at 30 mg/kg. RD 12,869 was reported to be effective in Cebus monkeys as well as in mice against S. mansoni, but ineffective in hamsters.[98]



(XXI)



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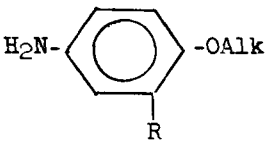
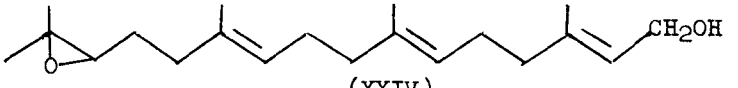


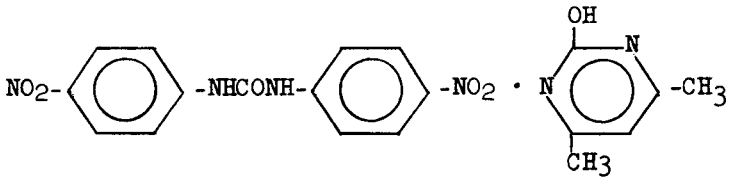
(XXIII)

The structure-antischistosomal activity relationship and retinotoxic effects of several series of nuclear substituted aminophenoxyalkanes (XXIII) was reported.[99] The diterpene, (-)-14,15-epoxygeranylgeraniol, (XXIV) applied to the tails of mice prevented schistosomiasis by reducing skin penetration by cercariae of S. mansoni.[100,101] A prolonged mild course of 2-4 mg/kg of body weight for 21 days of dehydroemetine given orally resulted in a high cure rate in a small number of cases with single or mixed infections with S. mansoni and S. haematobium.[102]

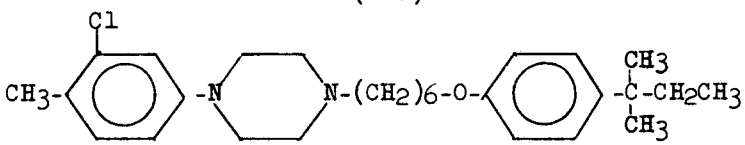


(XXIV)

The reproduction of S. mansoni in experimentally infected mice was suppressed with the coccidiostat, nicarbazin (an equimolar complex of 4,4-dinitrocarbanilide and 2-hydroxy-4,6-dimethylpyrimidine (XXV), when fed in the diet at dose levels of 0.2 to 1%. Withdrawal of the drug resulted in a resumption of egg laying by the worms.[103] Interest in nitrofuran derivatives and organophosphates continues.[104-109] The piperazine compound A 16,712 (XXVI) which is effective against S. mansoni in mice (25 mg/kg/day x 5), slightly effective in Cebus monkeys (500 and 1,000 mg/kg/day x 5) and ineffective in hamsters at a dose level of 1,000 mg/kg for 7 days, proved to be inactive in 10 patients up to the total dose level of 750 mg/kg.[110]



(XXV)



(XXVI)

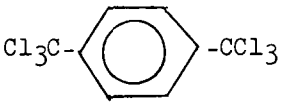
Other Trematode Infections - The drug, α,α,α,α',α',α'-hexachloro-p-xylene, (XXVII) which was shown to be promising in the treatment of clonorchiasis, was reported to have been withdrawn from clinical trials.[111]

Nematode Infections - Nematode parasites cause medically and economically important diseases in man and his domestic animals throughout the world. Among the more than 25 different worms that affect man, the large roundworm, the hookworm and the whipworm are responsible for more than half of mankind's helminthiases. The number of human helminthic infections in the United States has been estimated at about 44 million. The majority of these infections is due to pinworms (enterobiasis).

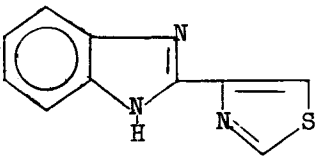
Thiabendazole (Mintezol®) (XXVIII) and bephenium hydroxynaphthoate (Alcopara®) (XXIX) are now available in the United States for the treatment of intestinal parasitoses. Thiabendazole is claimed to be particularly effective against enterobiasis and strongyloidiasis (threadworm disease). Bephenium is reported to be effective against hookworm infections.[1,5,7]

Tetramisole (XXX), a new broad-spectrum anthelmintic effective against a variety of gastrointestinal nematodes of domestic animals,[112] is under evaluation in man. Clinical trials against ascaris infections showed that a single dose of 5-6 mg/kg produced cures of 80% (8 of 10) and

88% (43 of 49). At this dose level minimal side effects were seen. No activity was reported for human hookworm, strongyloides or trichurid infections.[113,114] Optical isomers of tetramisole have been synthesized.[115] Although the dextro and levo forms are comparable in toxicity for mice, the levo isomer shows higher biological activity.[116] In vitro studies indicate that tetramisole causes muscular paralysis in ascaris worms. Specifically, the drug inhibits succinate dehydrogenase activity of the worm. The levo isomer is a more potent enzyme inhibitor than the dextro isomer or the racemic mixture.[117]



(XXVII)



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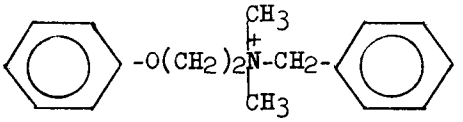
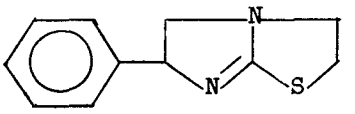


$C_{11}H_7O_3^-$

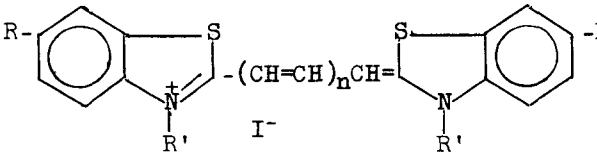
(XXIX)



(XXX)

Additional work has been reported on dymanthine, $C_{18}H_{37}N(CH_3)_2$, in 66 patients with multiple helminthic infections.[118] A 2.5-gram amount of the drug was given in 2 equally divided doses. Reduction in egg count was about 50% after two weeks for the ascarid, trichurid and hookworm infections. Side effects occurred in about half the patients.

Potential Chemotherapeutic Agents - Several anthelmintic compounds were reported in animal studies. Included are: Thiacyanines (XXXI) and hemithiacyanines (XXXII) related to dithiazinine (XXXIa);[119] methyl 5(6)-butyl-2-benzimidazolecarbamate (XXXIII);[120] 1,4-bis (2-diethylamino-ethoxy) anthraquinone dihydrochloride (XXIV) [121] and dithymyltrichlorethane (XXXV).[122]



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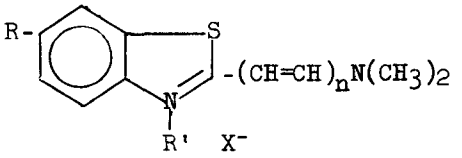
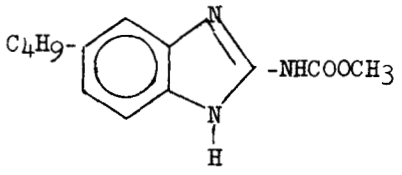
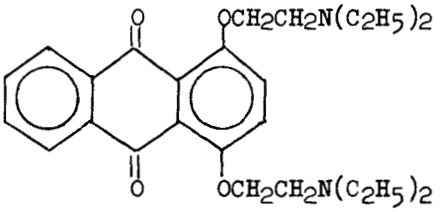


(XXXII)

a) R = H; R' = C_2H_5; n = 2

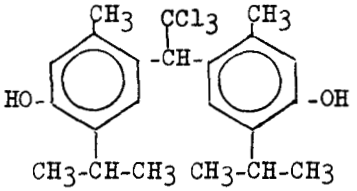


(XXXIII)



(XXXIV)

Cestode Infections - The action of the drugs quinacrine and niclosamide (Yomesan®) (XXXVI), employed in the treatment of tapeworm infections, on the metabolism of the rat tapeworm, Hymenolepis diminuta, was compared.[123] Niclosamide appears to have a greater effect on the process of energy production in tapeworms.



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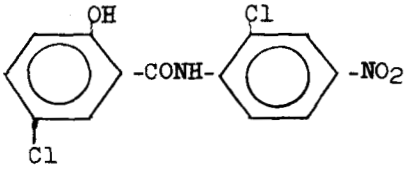
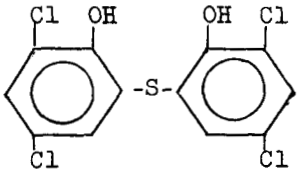
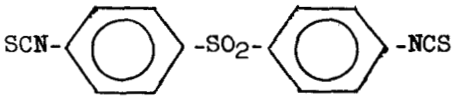


(XXXVI)



(XXXVII)



(XXXVIII)

Bithionol (XXXVII) administered orally in a single dose of 50-66 mg/kg body weight to 8 patients with Taenia saginata and to 8 others with Diphyllobothrium latum proved to be effective in eliminating the infections[124] with no serious side reactions. The in vivo activity of the diphenylsulfone (XXXVIII) against Hymenolepis nana in rats compares favorably with known cesticidal compounds.[125]

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Chapter 13(b). Animal Antiparasitic Agents

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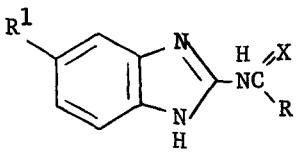
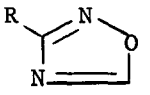
Anthelmintic Agents. A wide spectrum of activity against round worms in many host species was reported for compounds of formula I[1]. Activity is present in thiocarbamates (X = S, R = C_2H_5O), acyl derivatives (X = O, R = alkyl), carbamoyl derivatives (X = O, R = alkylamino). Activity is enhanced by alkyl groups at R^1. The compound of choice is SKF 29,044 (R^1 = n-butyl, X = O, R = OCH_3).



I

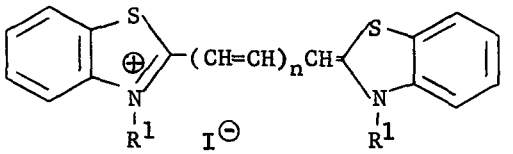
1,4-Bis(2-diethylaminoethoxy)anthraquinone dihydrochloride, RO2-9009, was reported[2] active in mice vs. H. nana, S. oblevata, and migrating ascarid larvae, but inactive vs. N. dubius. Compound uptake by the parasites was observed fluorimetrically but was not related to the removal of worms in vivo nor to their death in vitro. Comparisons were made with quinacrine, thiabendazole, piperazine, and trichlorophenol.

A series of 1,2,4-oxadiazoles of type II was reported active against nematodes in laboratory rodents.[3] The compound of choice had R = p-chlorophenyl. Compounds substituted in the 5-position and dihydro derivatives were inactive.

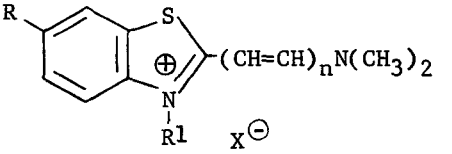


II

Preparation and testing of analogs of dithiazinine (III R^1 = C_2H_5, n = 2) with substituents in the 6-position of the benzothiazole ring, varying R^1 to methyl, and with n = 0, 1, 2, and 3 did not produce more active and/or less toxic compounds when tested against migrating A. suum larvae in mice.[8] Among compounds of the type IV activity against the larvae of A. suum was found in mice with doses of 10.5 or 2.5 mg/kg. at intervals of 4 hours. The compounds were uninteresting against nematodes of sheep.



III



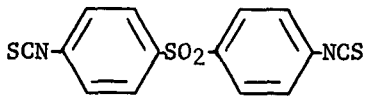
IV

Structural specificity was high as is shown in Table I for Type IV.

Table I

	R	R^1	n	X	Activity In A. suum in mice
XV	H	C_2H_5	1	I	Inactive
XVI	CH_3	CH_3	2	I	Very active
XVII	CH_3	C_2H_5	2	Cl	Inactive
XVIII	CH_3O	CH_3	2	I	Very active
XIX	C_2H_5O	CH_3	2	I	Active

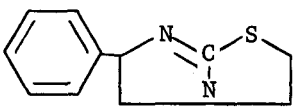
XVI and XVIII were effective in preventing lung damage in artificial infections by A. suum in swine when administered in the feed and also prevented liver lesions.



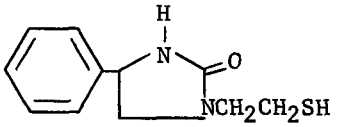
V

Compound V was reported active vs. immature H. nana in mice (10 mg/kg) and rats (100 mg/kg) and to possess a high margin of safety in these species.[9]

In the case of tetramisole, VI, it was reported[4,5] that anthelmintic activity is almost exclusively a property of the S(-) isomer and that the toxicity of the two optical isomers is equal. The relative safety of the S(-) is thus about twice that of the racemate. The Belgian workers established the absolute configurations of the isomers by synthesis from the optically active phenylethylenediamines.[4] The American workers resolved the racemate.[5] Studies by the Belgian workers on the mode of action of tetramisole led them to postulate interference with succinic dehydrogenase, possibly by the hydrolysis product, VII.



VI

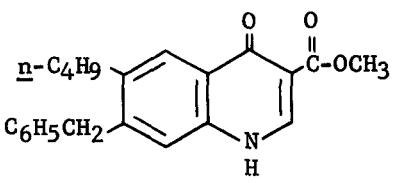


VII

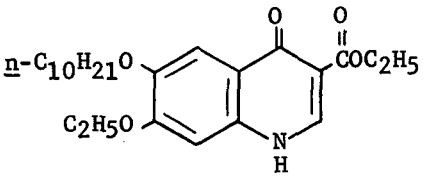
Reemphasis to the possible effects that physical form and purity may have on activity was provided by a study in which finer particle size and a higher purity are claimed to increase the activity of phenothiazine vs. relatively phenothiazine resistant strains of H. contortus.[7]

Anticoccidial Agents. Two extensive investigations have led to new compounds of reported high suppressive activity versus many strains of coccidia in the laboratory.

Workers at ICI published[10] on a series of which the compound of choice is methyl benzoquate VIII. May and Baker workers[11] confirmed the structure activity relationships reported for buquinolate and find an unexpected peak of activity in the structure, IX.



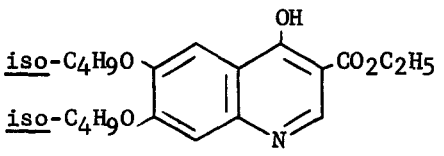
VIII



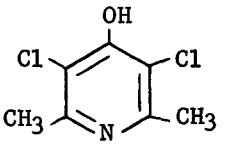
IX

There is high activity when the decyl chain is replaced by heptyl or octyl but this virtually disappears when the chain is smaller than heptyl or greater than undecyl.

An extensive study[12] on methyl benzoquate VIII, buquinolate X and



X

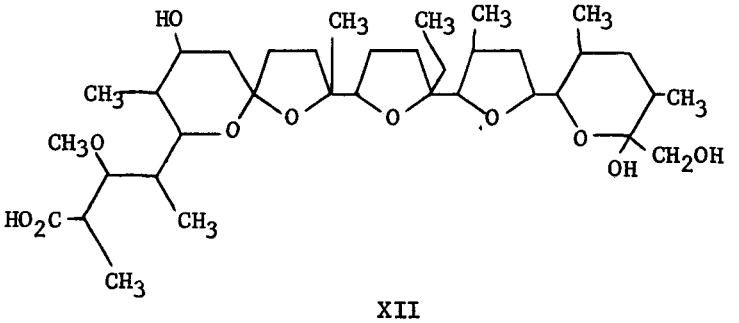


XI

metichlorpindol, XI, showed that all were active in suppressing sporozoite development at an early stage. Reversal experiments utilizing chick embryo infections and methyl benzoquate, VIII, were unsuccessful in finding an antagonist.

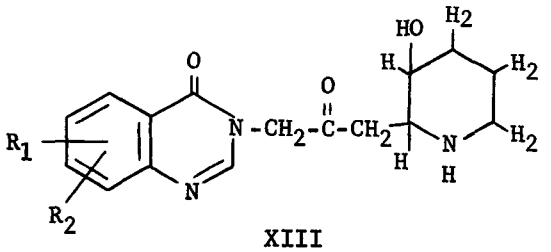
Monensin, a compound produced by Streptomyces cinnamonensis, is an inhibitor of alkali metal cation transport into rat liver mitochondria and is claimed to have broad spectrum anticoccidial activity.[13] The complete structure was determined by x-ray analysis of the silver salt.

The structure of monensin was shown to be XII.



XII

Very high (1-10 ppm of diet) prophylactic and therapeutic activity against 6 species of coccidia is claimed for substituted febrifugines of the general structure[14], XIII.



XIII

R_1 and R_2 are various combinations of halogen atoms and hydrogen. Derivatization of the hydroxyl and secondary amino functions of the piperidine ring reduced activity.

Histomonostats. A series of 5-nitroimidazoles of type XIV was reported[15] to be of high activity vs. Trichomonas foetus and Histomonas meleagridis. It was reported that 2-(p-fluorophenyl)-1-(2-hydroxyethyl)-5-nitroimidazole (I, $R_1 = HOCH_2CH_2$, $R_2 = p\text{-}FC_6H_4$), about 3 times metronidazole (I, $R_1 = HOCH_2CH_2$, $R_2 = CH_3$) experimentally vs. T. foetus is undergoing clinical tests. The compound of choice in the series for histomoniasis is 1-methyl-2-carbamoyloxymethyl-5-nitroimidazole (I, $R_1 = CH_3$, $R_2 = H_2NCOCH_2$-), experimentally 4-8 times as active as dimetridazole (I, $R_1 = R_2 = -CH_3$).

XIV

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Chapter 14. Antifungal Agents

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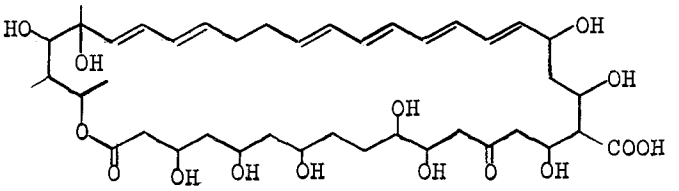
This review is essentially a continuation of the same chapter in the 1966 Annual Reports.

Polyene Antibiotics - This continues to be the most important class of compounds for the treatment of systemic mycoses and conditions caused by Candida.

Amphotericin B has been found useful in treatment of the following diseases not previously mentioned in the last report: rhinocerebral phycomycosis,[1] rhino-orbital mucormycosis,[2] chromoblastomycosis,[3] and mycotic endocarditis due to Cryptococcus neoformans.[4] The degree of susceptibility of a variety of Cryptococcus neoformans strains to amphotericin B was shown by an in vitro study in which 65 human and 25 environmental isolates of C.n. were treated with various concentrations of the antibiotic.[5] The minimum inhibitory concentrations ranged from 0.024 µg/ml to 0.39 µg/ml.

A statement that 20% of Candida strains are resistant to nystatin[6] was most emphatically refuted by several authors[7,8,9,10,11] who are in agreement that natural resistance to polyene antibiotics has never been demonstrated. Thus other causes must be sought for patient resistance to nystatin therapy. The structure of nystatin is almost solved. Structure I has been assigned to nystatin aglycone[12] thus leaving unknown only the site of attachment of the sugar, mycosamine.



I

Evidence that hamycin will be a useful antifungal drug continues to appear. In two studies[13,14] covering over 200 cases of mucocutaneous candidiasis due to Candida albicans a cure rate of 83% was obtained. In another study[15,16] 2 cases of histoplasmosis and 4 cases of blastomycosis treated with micronized hamycin showed objective improvement and the dis-

appearance of the causative organism. Since hamycin seems to be less toxic than alternative methods of therapy further work with this drug is most certainly warranted. A single case of bronchopulmonary aspergillosis due to A. fumigatus also exhibited a clinical cure after hamycin treatment.[17]

The mode of action of polyene antibiotics continues to be the subject of a number of reports.[18,19,20] It begins to appear that polyene antibiotics interact somewhat indiscriminately with membranes common to microorganisms and to host. Thus recent evidence[18] confirms the fact that lysosome membranes are disrupted in such a way as to release hydrolytic enzymes. The lysosomes from various organs display different susceptibilities and furthermore the smaller polyenes induce more drastic changes than the larger ones. However, amphotericin B, a larger polyene, causes considerable disruption of kidney lysosomes particularly at acid pH. It is conceivable that this plays a role in the renal lesions induced by this drug and may also be related to pyrogenic responses to the drug.

The possibilities of applying proton transfer reactions to differentiation of polyene antibiotics has been examined and claimed to be useful.[21]

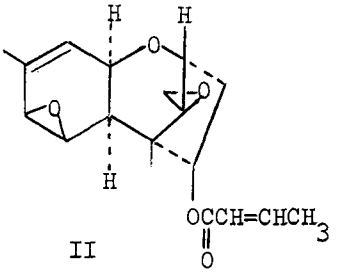
A mode of action study of azalomycin F on Candida albicans indicates that at its minimum growth inhibitory concentration the antibiotic strongly inhibited amino acid incorporation into cellular protein and prevented generally the substrate respiration of amino acids.[22] This same article contains a brief bibliography of mode of action studies on antifungal antibiotics.[22]

Aureofungin[23,24] and champamycin B[25] are newer members of this class which are potentially interesting.

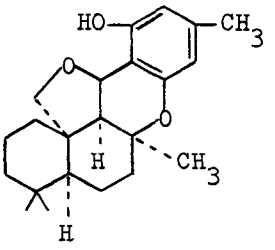
Nonpolyene Antibiotics - In an effort to obtain an increased concentration of griseofulvin in the blood the 4'-oxime and 4'-alcohol derivatives were prepared and then tested in rabbits.[26] After intravenous injection they were quickly converted to griseofulvin. Oral dosing gave plasma levels of griseofulvin as high as or higher than when the antibiotic itself was used but the high levels were of short duration.

Two reports[27,28] have drawn attention to the fact that griseofulvin has been shown to induce liver tumors when administered orally to young mice. This would seem to suggest the need for epidemiologic investigations following human therapy with griseofulvin. Meanwhile it is suggested[27] that the drug probably should not be used where some other drug would be effective. In a more optimistic vein two other reports[29,30] indicate that although oral griseofulvin in mice routinely causes a rather high degree of abnormal porphyrin metabolism, in humans only 2 out of 10 patients were affected.

Structure II has been proposed[31] for crotocin, an antibiotic which is probably of limited medicinal use but which is a member of the sesquiterpenoid-ester group of antibiotics. Two potentially useful antibiotics with high in vitro activities are siccanin[32] (III) and leucinamycin.[33]



II

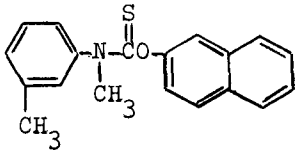


III

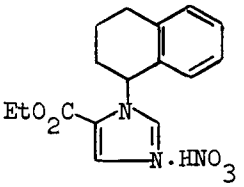
Review articles on the following subjects will be of varying usefulness: griseofulvin,[34] pimaricin,[35] mechanism of action,[22,36] and official microbiological assays.[37]

Synthetic Antifungal Agents - A critical review and evaluation of the laboratory investigations and clinical uses of the recently introduced antifungal drug tolnaftate (IV) has appeared.[38]

High in vitro and in vivo activity has been reported[39] for 1-(1,2,3,4-tetrahydro-1-naphthyl-5-(ethoxycarbonyl) imidazole nitrate (V).



IV



V

Otomycosis, a primary fungal infection of the ear, caused by A. niger, was most effectively treated with 5-chloro-7-iodo-8-hydroxyquinoline.[40]

N-Hydroxy-N'-methylthiourea (Noxythiolin) has proven clinically effective in treating vaginal Candida infections.[41]

Varying degrees of in vitro antifungal activity have been claimed for a variety of chemical types. Among these are substituted 8-hydroxyquinolines,[42] acetylenic compounds,[43] 2-fluorofatty acids,[44] alkyl phenyl sulfides,[45] aryl thiocyanates,[46,47] substituted phenylhydrazines and phenylpyrazolones,[48] methyl-5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxylate,[49] p-hydroxypropiophenone,[50] substituted benzimidazoles,[51] 2,4-bis(arylamino)-5-methylpyrimidines[52] and isothiocyanates.[53]

A detailed description of the procedure to be followed in developing an antifungal drug from initial in vitro studies to clinical investigations in humans is available.[54]

Several reviews dealing with the nature and treatment of mycoses have

appeared during the past year.[55,56,57]

An up-to-date listing of the antifungal agents used in medicine can be found in the 1967 edition of the Handbook of Pharmacology.[58] Amplification of the uses and properties of a number of these appears elsewhere.[59]

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Chapter 15. Antineoplastic Agents

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Introduction - Successful chemotherapeutic treatment of cancer requires the existence of an exploitable metabolic difference between the offending neoplastic cells and all cells, essential to the integrity of the organism, that are to be exposed to the therapeutic agent. It is clear that such metabolic quirks on the part of neoplastic cells do exist in some varieties of cancer in man. This is amply demonstrated by the estimated cure rate of 70% achieved by chemotherapy in women with trophoblastic cancer, as well as by the predictable palliation given to patients with Wilms' tumor, lymphoblastic leukemia or Hodgkin's disease. When examined in this context the work published during 1967 records one major advance, the demonstration that some human cancer cells are dependent for continuing proliferation and viability upon the presence of exogenous asparagine. By using the enzyme L-asparaginase to destroy the asparagine normally present in blood and extracellular fluids at least one type of human cancer can be caused to regress.

Asparaginase - Oettgen and coworkers have now demonstrated that the enzyme L-asparagine amidohydrolase (E.C. 3.5.1.1), extracted from E. coli, possesses clinical activity against some forms of human cancer.[1] The antitumor activity of asparaginase was first described by Kidd as an unknown principal in guinea-pig serum;[2] this was identified as the enzyme L-asparaginase by Broome.[3] Sensitivity to asparaginase equates with a metabolic dependence of the cells in question upon exogenous asparagine for normal synthesis of RNA and protein and for the maintenance of viability. Asparagine dependence is not uncommon in murine leukemias;[4] it also occurs in tumors of the rat,[5] dog,[6] and man.[1] Cells of tumors resistant to asparaginase and normal cells do not apparently require exogenous asparagine. This dependence permits the use of an *in vitro* test of tumor cell sensitivity wherein cellular incorporation of labeled uridine and/or valine into RNA and protein respectively is measured in the presence and absence of asparagine and asparaginase.[1] In their initial publication Oettgen et al. have recorded significant therapeutic alterations in four patients with acute lymphoblastic leukemia, and in one with acute myelocytic leukemia. Two patients with lymphosarcoma and two with acute myelogenous leukemia did not respond. They observed a positive correlation between therapeutic response and the requirement of the tumor cells for L-asparagine *in vitro*. The available preparation produces side effects of chills, fever, nausea and vomiting. These are self-limited and may relate to residual non-enzymic bacterial impurities. Hypersensitivity to this foreign protein was observed in some patients but did not, in itself, necessitate discontinuance of therapy. The development of clinical resistance to asparaginase was observed; this correlated with a loss of asparagine dependence in the *in vitro* test.

Alkylating agents - 1,3-bis(2-chloroethyl)-1-nitrosurea, (BCNU), (I) originally attracted investigative interest because of its ability to cross the blood-brain barrier and because it was highly effective against L-1210 leukemia implanted intra-cerebrally in mice; it is now being tested extensively in the management of Hodgkin's disease. Lessner has reported clinical benefit in 15 of 30 patients with far-advanced Hodgkin's disease, previously treated with radiation and a variety of chemotherapeutic agents.[7] The drug produces both marrow and liver damage in animals but only marrow toxicity has been seen in current studies. Preclinical studies presently center upon the potential efficacy of other derivatives and the mechanism of drug action. Johnston and Opliger have synthesized a series of nitroso derivatives of biurets, biureas and carboxamides.[8] They observed that while some of these (e.g. II, III, IV) had antitumor activity, substitution by the 2-chloroethyl group (III, IV) did not result in the outstanding activity against L-1210 leukemia obtained with BCNU and related nitrosoureas. Because of cross resistance between BCNU and alkylating agents in a hamster plasmacytoma and in microorganisms and a similarity of the biochemical effects of this drug to those of alkylating agents it has been included in this class of drugs. The structural resemblance to a nitrogen mustard is misleading in this context since there is good evidence that BCNU does not alkylate through its -carbons,[9] nor are diazoalkanes formed in aquaeous solutions at physiologic pH.[9,10] Recent studies on the physiologic disposition of labeled BCNU in man and animals have been reported.[11]

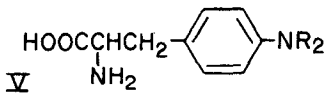
$R=CH_2CH_2Cl$

I $RNCONHR$ (NO)

II $CH_3NCONHCONH_2$ (NO)

III $RNCONHCONHR$ (NO)

IV $RNCO(CH_2)_4CONR$ (NO, NO)



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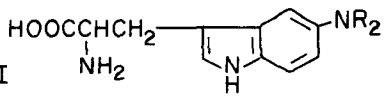
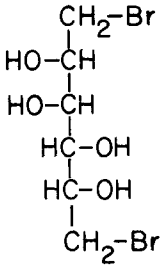


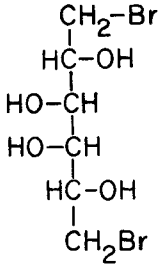
VI

The possibility that selective cytotoxicity may be obtained by structural modifications of alkylating agents continues to attract investigative interest. Recent reports give new affirmation of the clinical utility of L-phenylalanine mustard, (melphalan) (V) in multiple myeloma,[12] and record the activity of tryptophane mustard (VI) in the same disorder.[13] The two drugs also show similarities in their physiologic distribution in tumor-bearing animals.[14]

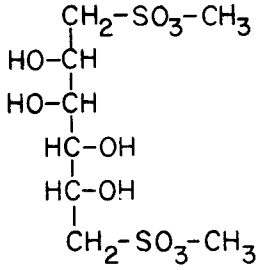
Institoris and coworkers[15] have continued their explorations of the pharmacology of dibromomannitol (VII), dibromodulcitol (VIII), and 1,6-dimesyl-mannitol (IX). These drugs are useful in the management of chronic myelogenous leukemia although they have not been shown to possess advantages over busulfan (X) itself. The two dibromohexitols are alkylating agents and produce biochemical lesions in cellular metabolism



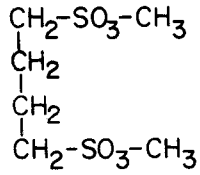
VII



VIII



IX



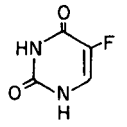
X

similar to those of alkylating compounds; however, dibromoalkanes (C=4 or 6) which possess the same alkylating capacity are ineffective in cytostatic assay. Furthermore, the bond between carbon and bromine is more stable in VII and VIII than the bond of the functional groups in the known alkylating agents. The half-time of degradation of VII and VIII in vivo is between 5 and 10 hours; their metabolites include derivatives with bromine in a covalent bond.

A recently published symposium containing reports of clinical and biochemical studies on cyclophosphamide merits attention.[16]

Pyrimidine and Purine Analogues - Perhaps the most interesting development in this area has been the emergence of topical 5-fluorouracil (FU) (XI) as a useful therapeutic agent in the management of multiple superficial basal cell carcinomas.[17] While it is generally accepted that a single basal cell carcinoma is still managed most effectively by surgical excision, topical fluorouracil is being tested in patients with multiple recurrent lesions where surgery is not practical. The drug produces erythema, edema and necrosis of the visible tumor and the skin immediately adjoining it. Following resolution of the inflammation, healing is reported, without apparent residual cancer, in better than 80% of lesions. The duration of control thus achieved and the utility of repeated courses of therapy remain to be determined. Even when the agent has been applied over wide areas systemic manifestations of fluorouracil effect have not been noted. Normal skin is reported to be relatively resistant to the inflammatory effect of topical FU.

Synthesis of derivatives of XI and of 5-fluoro-2'-deoxyuridine (XII) continues in a search for compounds that will be active, less susceptible to degradation by nucleoside phosphorylase and phosphorylated



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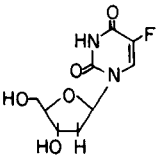
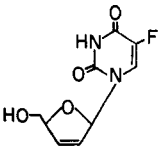
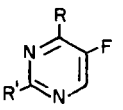


XII



XIII



XIV

	R	R'
A	OH	H
B	H	OH

by a mechanism not involving thymidine kinase. Khwaja and Heidelberger have reported the synthesis of 1-(2,3-dideoxy-2,3-didehydio-β-D-glycero-pentofuranosyl)-5-fluorouracil (XIII).[18] They found that it was not a substrate for nucleoside phosphorylase, uridine kinase, or thymidine kinase. In cell culture and in experimental animals the compound inhibited growth of several tumor lines including two resistant to XII. In this same context Sartorelli and Creasy[19] reported weak inhibitory activity of 4-hydroxy-5-fluoropyrimidine (XIVA) and 2-hydroxy-5-fluoropyrimidine (XIVB) in tests on growth and DNA synthesis by sarcoma 180 ascites cells. They confirmed that the activity of XIVA is mediated by a conversion of that compound to XI by the enzyme xanthine oxidase.

The activity of 1-β-D-arabinofuranosyl cytidine (ara-C or CA) against leukemia in man led Wechter[20] to synthesize various nucleotides of CA and dinucleoside phosphates of the type CApX and XpCA where X represents a second nucleoside. The simple nucleotides were cytotoxic; dinucleoside phosphates with a 3'→5' linkage were more active than those of 2'→5' configuration.[21] Mouse leukemia cells resistant to ara-C were also resistant to its phosphorylated derivatives.

Of the numerous new purine analogues under active study in cell culture or animal tumor systems only 6-methylmercaptopurine riboside (6MMPR) has received significant clinical trial during the past year. The original hope that 6MMPR would be inhibitory *in vivo* to 6-mercaptopurine (6MP) resistant tumors has not been realized;[22,23] however, 6MMPR and 6MP are reported to have synergistic effects against Ehrlich ascites tumor[22] and L-1210 leukemia[23] in mice. In its initial clinical trials this drug combination has produced a complete remission in 7 of 22 adequately treated patients with acute myelogenous leukemia,[24] a response rate somewhat higher than that usually obtained with 6MP alone.

Miscellany - Hydroxyurea, an agent of established utility in the management of chronic myelogenous leukemia, continues to receive study as a biochemical tool. Studies with purified enzymes from *E. coli*[25] and

Novikoff hepatoma[26] and on drug induced changes in deoxyribonucleotide pools in intact E. coli [27] have verified its inhibitory effect on reduction of ribonucleotides. Extensive observations on the structure-activity requirements for the drug-induced inhibition of DNA synthesis have been reported.[28] The cytotoxic consequences of the drug's inhibitory effects on DNA synthesis differ markedly for different lines in cell culture,[29,30] and for regenerating liver, bone marrow and intestinal crypt cells in the rat.[31] These observations emphasize that inhibition of a vital process within a cell may be tolerated for many hours without lethal damage to that cell. Hydroxyurea is also being studied as a radiation potentiating agent.[32,33]

Geller and coworkers[34] have reported benificial effects in patients with advanced carcinoma of the prostate treated with each of three progestational agents: hydroxyprogesterone caproate, chlormadinone acetate and cyproterone acetate. These therapeutic effects were obtained without evidence of feminization.

During 1967 an excellent textbook[35] and two symposia[36,37] on general aspects of cancer chemotherapy have been published; the text, in particular, along with the volume which preceded it, will serve as a useful reference work for those interested in this field.

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Section IV - Metabolic Diseases and Endocrine Function

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Chapter 16. Antidiabetics

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It is generally conceded, that the tendency to diabetes is inherited but its mode of inheritance is unknown.[1,2] The lack of a reliable marker for prediabetes adds to the difficulty of determining the genetic mode of its transmission. Several characteristic abnormalities have been observed in prediabetics (vascular changes, excessive synalbumin levels, abnormal ACTH secretion, increased growth hormone levels, and delayed and diminished insulin response to glucose load), but none has been accepted as a characterization of the prediabetic state. On the opposite end of the spectrum, very little is known about the cause of the complications of diabetes. Diabetics are subject to abnormalities of the large blood vessels, thus increasing the likelihood of cerebrovascular, coronary, and peripheral vascular disease. Microangiopathy, another complication of diabetes, may lead to diabetic retinopathy or glomerulosclerosis. The relationship between the metabolic defects and the complications is poorly understood.[3,4] Evidence suggests that control of the metabolic disorders of diabetes will decrease the probability that complications will occur.[4]

Diabetes is characterized by a deficiency of effective insulin which could result from the defective synthesis or release of insulin by the pancreatic β-cells, an increased hepatic degradation of insulin by glutathione-insulin transhydrogenase, or the binding of insulin to a larger protein in the bloodstream. Vascular abnormalities may inhibit its exit from the bloodstream. The deficiency of effective insulin might also be explained by tissue insensitivity to insulin or insulin antagonism by hormones or other substances. There is not sufficient evidence to implicate any one of these defects as the primary metabolic lesion of diabetes.

Oral hypoglycemics have been used effectively for over 12 years. Since these drugs (sulfonylurea and biguanide derivatives) do not correct all of the metabolic disorders of diabetes and since they do have a number of limitations, hundreds of new compounds have been synthesized and tested for hypoglycemic activity. As stated by Dr. Rachmiel Levine, "the sulfonylureas deserve great attention because they stimulated the investigators even more than they did the beta cells". The only useful compounds to result from this work, however, are analogs or derivatives of the earlier ones and offer no particular advantages over the original ones, other than increased potency and duration of action. When more is known about the causes and the basic biochemical abnormalities of diabetes it will undoubtedly be possible for the medicinal chemist to design compounds to

more effectively treat or prevent the development of diabetes. Consequently, the major portion of this review is devoted to the discussion of current basic research in diabetes rather than to an encyclopedic classification of hypoglycemic compounds.

Insulin

Chemical synthesis. Improved techniques in protein synthesis make it appear likely that appreciable quantities of synthetic insulins will be available for investigational purposes in the not too distant future.[5] Until recently the major problem in insulin synthesis has been the final combination step where only very low yields were obtained, even when using natural chain preparations. Claims have been made of yields of up to 50% for the combination step but they were based on the specific activity of the final product rather than on the amounts of the starting chains.[6,7] Recent reports indicate that the yields of insulin are substantially higher when the sulfhydryl form of one chain is combined with the S-sulfonated derivative of the other chain.[8] Yields of 60-70% and 12-22% have been reported for the synthesis of insulin from natural and synthetic chains respectively. These yields are based on the amounts of starting chains used. The availability of synthetic insulins will provide for methods of studying the relationship between structure and biological activity. The synthesis of insulins from radioactive amino acids will open the way for studying the distribution of insulin in the body, its site of action, and its mode and rate of degradation.

Biosynthesis. Recent evidence supports the existence of a "proinsulin".[9] Human islet cell tumor tissue and isolated rat islets initially incorporated radioactive amino acids into a high molecular weight protein which contained the amino acid sequence of insulin and reacted with insulin antibodies. After additional incubation, the radioactivity was transferred to a fraction corresponding to insulin.[9] Insulin was liberated from the larger protein by incubation with trypsin.[9] Bovine "proinsulin" has a molecular weight of about 8,300 and has only one N-terminal and one C-terminal amino acid (phenylalanine and asparagine, respectively).[10] It is a linear molecule composed of insulin B chain (N-terminal) and insulin A chain (C-terminal) which are joined by a heptacosapeptide.[10] The amino acid composition of the "connecting peptide" is known and studies on its amino acid sequence are virtually complete.[10] Insulin is apparently liberated from "proinsulin" by proteolytic cleavage of an Ala-Arg bond in position 30-31 and an Arg-Gly bond in position 57-58 of "proinsulin".[10] The disulfide bonds are intact at the time of cleavage. The conversion of "proinsulin" to insulin is analogous to the proteolytic cleavage of proenzymes to produce enzymes. This hormone precursor might be synthesized initially in order to protect the insulin molecule or to facilitate its transport within the β-cell.[9] It may also be important because of its ability to orient the A and B chains for disulfide bond formation.[10] The fact that "proinsulin" is immunoreactive[9] and exhibits some biological activity in adipose tissue *in vitro*[10] suggests that it could be a complicating factor in determining plasma insulin levels.

Diabetic Insulin. The concept that an abnormal insulin may occur in some diabetics has been the subject of much speculation but very little evidence has been presented to support this view. One study demonstrated that serum insulin from juvenile diabetics is more resistant to insulinase than insulin from normal subjects.[11] The difference in degradation rates could be due to structural differences between the two insulins but no chemical evidence is presented to substantiate such a proposal. It has been reported that normal pancreatic insulin causes a greater stimulation of glucose conversion into glycogen in the rat diaphragm than pancreatic insulin from diabetics.[12] A recent paper reports the amino acid composition of some normal and diabetic insulins.[13] Diabetic pancreatic insulin had the expected amino acid composition in all cases but one. The remaining sample was slightly deficient in isoleucine and contained slightly more lysine than normal insulin but the differences were not great and are probably within the experimental error of a standard amino acid analysis. It must be concluded that the presence of an abnormal insulin in diabetics has not been established.

Release. Studies on the mechanisms of insulin secretion have been aided in recent years by the development of *in vitro* methods of measuring insulin secretion by the perfused pancreas[14,15], isolated pancreatic tissue[16], and isolated intact pancreatic islets.[17] These methods, in conjunction with improved immunoassay techniques, have provided a vast amount of information concerning substances, both physiological and pharmacological, which stimulate insulin release.

Recent evidence suggests that certain gastrointestinal hormones (secretin, pancreozymin and gastrin) promote insulin secretion.[18] This may explain why oral glucose is a better stimulator of insulin release than i.v. glucose.[18] The physiological significance of this phenomenon has yet to be established but it could be a means of preventing the high substrate concentrations and hyperglycemia that would otherwise follow the ingestion of a large meal. That glucose acts directly on the β-cell to stimulate insulin release almost immediately is well established.[19] Whether it is glucose or one of its metabolites which stimulates insulin release remains to be determined. Insulin secretion *in vivo* is also promoted by mannose, fructose and other utilizable sugars. Glucose-induced insulin release is blocked by 2-deoxyglucose[20] and by mannoheptulose.[21]

Several amino acids and proteins also stimulate insulin release.[22] The mechanism by which the various amino acids cause insulin release is apparently different since diazoxide inhibits the insulin releasing effect of leucine but not of arginine.[23] Also, leucine-induced insulin release is magnified by pretreatment with sulfonylureas whereas this is not the case with other amino acids.[23] Arginine infusion has been shown to increase blood sugar levels initially[24] but it is unlikely that this initial increase in blood sugar is of sufficient magnitude to stimulate the release of the large quantities of insulin observed in these experiments. Arginine infusion also stimulates the secretion of growth hormone[25,26] but it is not known how this effect is releated to its insulin releasing activity. The fact that elevated plasma amino acid levels promote insulin

secretion thereby increasing the transfer of amino acids across cell membranes and increasing the rate of protein synthesis seems quite logical. One wonders, however, if the intervenous infusion of 30 grams of arginine can be considered to be a physiological stimulus!

Free fatty acids (FFA) and glycerides promote insulin release.[27] This effect is not mediated by glucose but may be dependent on the conversion of the FFA to ketone bodies which are known to stimulate insulin release.[28]

Insulin release is stimulated by a variety of hormones. Glucagon and ACTH induce insulin release *in vitro* and *in vivo* by a mechanism which may be related to their effect on intracellular concentrations of cyclic AMP.[14,29] Glucocorticoids and growth hormone affect insulin by increasing the sensitivity of the β-cells to stimulation by glucose.[30,31]

The pancreatic islets are supplied with both adrenergic and cholinergic nerve endings.[32] Insulin secretion *in vitro* is inhibited by adrenergic drugs[33] and the effect is blocked by alpha-adrenergic blocking agents but not by beta-adrenergic blocking agents.[33] Stimulation of the β-receptors with isoproterenol increases plasma insulin levels in humans.[34] Cyclic AMP, which produces the same effects as stimulation of the beta receptors, also promotes insulin release. Vagal stimulation[35] and cholinergic drugs[33] promote insulin secretion and the effect is blocked by atropine. The autonomic nervous system may play an important role in the regulation of insulin secretion but more research is necessary before the exact nature of this role can be determined.

Insulin secretion is inhibited by thiazide diuretics and by diazoxide, an antihypertensive, nondiuretic benzothiazine[36], which has been used to treat certain types of hypoglycemia.[37]

The onset of diabetes is accompanied by changes in β-cell sensitivity to glycemic stimulation. Recent studies have shown that the insulin response to glucose is slower in mild diabetics than in normal subjects.[38] The mild diabetics in this study secreted a greater quantity of insulin in response to a given glucose load but this was probably because of the prolonged stimulation caused by the persistent hyperglycemia.[38] The moderate diabetics did not show a marked insulin release in response to glucose despite the existence of hyperglycemia.[38] This emphasizes the need for further study of the mechanisms of insulin secretion as a means of learning more about the metabolic lesions of diabetes.

Metabolic Effects. The effects of insulin on the metabolism of carbohydrates, proteins and lipids in muscle, adipose tissue and the liver are well documented elsewhere[39-41] and will not be discussed here.

Serum ILA. All of the insulin-like activity (ILA) of serum cannot be neutralized by insulin antibodies.[42] This has stimulated a great deal of research on the nature of serum factors which exhibit ILA in bioassays but are not immunoreactive and, hence, are not insulin.[43] One group has designated the forms of serum ILA as "free" insulin (immunoreactive and biologically active in muscle and adipose tissue *in vitro*) and "bound" insulin (biologically active in adipose tissue *in vitro* but not immunoreactive).[44] The forms of serum ILA have also been classified as "typical" and "atypical" insulin.[45] Both exhibit ILA in rat adipose tissue *in vitro* but only

"typical" insulin is supressed by insulin antibodies.[45] Others have referred to the two forms as "supressible" and "nonsupressible" ILA.[46] Both are biologically active in adipose tissue in vitro but only the former is neutralized by insulin antibodies. Another form has been described as "total serum insulin activity" (TSIA).[47] It stimulates glycogen synthesis in muscle but does not represent true insulin.[48] Others have studied a form of ILA which can be extracted from serum along with albumin by alcoholic trichloroacetic acid.[49] This substance is biologically active in a number of systems but is not supressed by insulin antibodies.[50] The term "non-supressible insulin-like activity" (NSILA) has been adopted to designate this material.

"Free" insulin, "typical" insulin and "supressible" insulin are probably identical with pancreatic insulin but the nature of "bound" insulin, "atypical" insulin, TSIA, and NSILA is unclear. Various forms of ILA have been extracted from serum and partially purified.[48,51-53] The approximate molecular weights of the various forms have been reported as: "bound" insulin 60,000-100,000, "atypical" insulin 30,000, and NSILA 80,000-160,000.[48] These forms of ILA are similar with respect to electrophoretic mobility and behavior on anion and cation exchange resin.[48] This has led to speculation that they might be related or, indeed, identical.[48,54]

Whether "bound" insulin stimulates glucose uptake by muscle in vitro is controversial. One group has reported that it is inactive in muscle in vitro but can be activated by incubation with an adipose tissue extract (ATE).[44] Others, however, have found that "bound" insulin itself is active in muscle in vitro.[48] Other forms of serum ILA also promote glucose uptake by the rat hemidiaphragm in the absence of ATE.[49] It has been reported that serum levels of "bound" insulin decrease after glucose ingestion, a phenomenon which has been attributed to the conversion of "bound" insulin to "free" insulin.[44] It seems somewhat presumptuous to propose that "bound" insulin could be converted to "free" insulin when there is no convincing evidence to show that "bound" insulin is structurally related to or derived from pancreatic insulin. Serum levels of NSILA do not change after glucose ingestion[45,46] and don't appear to be related to the metabolic state of the individual.[55] It is doubtful that the non-supressible forms of ILA are of pancreatic origin since porcine insulin is not converted to NSILA by the isolated perfused liver.[55] The physiological role (if any) of serum ILA cannot be determined until more is known about its chemical and biological characteristics. The physiological significance of "bound" insulin is questionable since it possesses activity in vivo.[56]

Synalbumin

Diabetic plasma albumin has a greater capacity to antagonize insulin than normal albumin.[57] This led Vallance-Owen[57] to propose that this insulin antagonism might be related to the pathogenesis of diabetes. Others have also found that human albumin antagonizes insulin in the rat hemidiaphragm[58-61] and in other in vitro systems[61-63] but the physiological significance of this insulin antagonist has been questioned. A number of difficulties have been encountered while attempting to measure insulin antagonism associated with plasma albumin. One of the most serious

problems is the apparent variation between different albumin preparations. The situation is further complicated by the presence of an artifactual antagonist in Debro albumin.[65,66] The artifactual antagonist probably results from the dialysis procedure or the TCA-ethanol extraction of albumin from plasma. It is unlikely that this is the only antagonist present in Debro albumin[67] since: (1) Cohn Fraction V albumin, which is not dialyzed, is antagonistic[58,59,62], (2) an albumin preparation isolated by precipitation with a quaternary ammonium compound is antagonistic and its inhibitory activity does not change after dialysis[68], (3) human serum antagonizes insulin *in vitro*[68], (4) there are quantitative differences in antagonism between albumin from normal and diabetic subjects[57], and (5) there are variations in levels of synalbumin antagonism during glucose tolerance tests.[69]

It has been postulated that the synalbumin insulin antagonist might actually be the B chain of insulin.[70] The antagonist has been separated from albumin and has been shown to be similar to insulin B chain in many of its physicochemical properties.[71-73] The finding that albumin bound B chain antagonizes insulin *in vitro*[73-75] and *in vivo*[74] adds credence to the hypothesis that synalbumin and insulin B chain could be identical. The detection of immunoassayable B chain in human plasma further supports the B chain-synalbumin theory.[76,77]

Conflicting reports from *in vivo* experiments do little to alleviate confusion. One group[78] has reported that both synalbumin and an albumin-B chain complex antagonize the effect of insulin on muscle but not on adipose tissue in vivo while others[79] were unable to demonstrate any effect from the infusion of antagonistic albumin into intact rats. It should be mentioned that the first group used Debro albumin whereas the second study was carried out with Cohn Fraction V albumin. Debro albumin is almost always more highly antagonistic than Cohn Fraction V when assayed by the rat hemidiaphragm assay but it is questionable as to how much of this antagonism is real.

It seems reasonable to conclude that a real antagonist does exist. Whether it is actually the B chain of insulin is not known but this reviewer is unwilling to abandon the possibility that the two may be identical. Its physiological role (if any) has not been determined. A more reliable method of extracting this antagonist from plasma is needed. Further chemical characterization of the purified antagonist is necessary but this will undoubtedly prove to be a rather formidable task in view of the minute quantities of material available.

Biochemical Considerations

Gluconeogenesis is important in controlling blood sugar levels in normal and diabetic subjects.[80] Gluconeogenesis is not simply a reversal of glycolysis since there are barriers which obstruct the reversal of glycolysis between: (1) pyruvate and phosphoenolpyruvate, (2) fructose-1,6-diphosphate and fructose-6-phosphate, and (3) glucose-6-phosphate and glucose[80] (Fig. 1). These energetically disfavored reactions are circumvented by alternative reactions in gluconeogenesis. The alternative reactions are catalyzed by (1) pyruvate carboxylase and phosphoenolpyruvate carboxylase, (2) fructose-1,6-diphosphatase, and (3) glucose-6-phosphatase

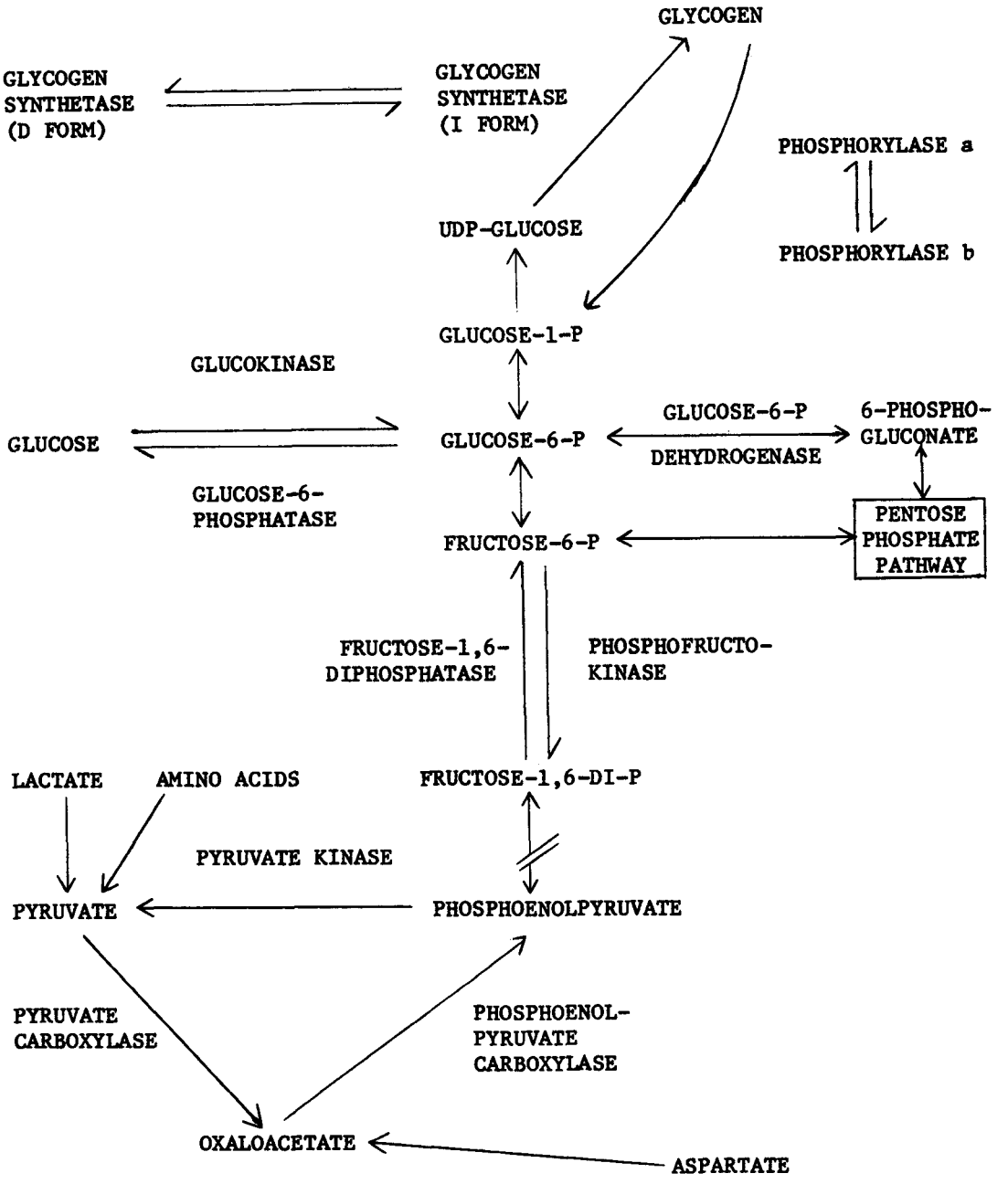

Figure 1. SUMMARY OF METABOLIC PATHWAYS

which are considered to be the key enzymes in gluconeogenesis.[80] Factors which promote gluconeogenesis increase the levels of these enzymes.[81] Levels of these enzymes are increased in alloxan-diabetic animals and in animals treated with anti-insulin serum (AIS).[81,82] Insulin restores gluconeogenetic enzyme levels to normal in these animals.[81] Utilizable sugars depress the elevated gluconeogenetic enzyme levels in fasted rats but not in AIS treated rats.[82] This indicates that the depression of gluconeogenetic enzyme levels is related to the metabolism of carbohydrates. Levels of key glycolytic enzymes (glucokinase, phosphofructokinase, and pyruvate kinase) (Fig. 1) are depressed in alloxan-diabetic animals.[81] Enzyme levels are normalized by insulin treatment.[81] Insulin therefore stimulates glycolysis and inhibits gluconeogenesis.

Another enzyme of current interest is glucose-6-phosphate dehydrogenase, which catalyzes the first step in the pentose phosphate cycle (Fig. 1). This pathway accounts for a high proportion of the glucose metabolized in adipose tissue and for some of the glucose metabolism in other tissues.[83] An important function of this pathway is the production of NADPH which is used as an energy source for the biosynthesis of lipids[84] and other materials.[85] Glucose-6-phosphate dehydrogenase may affect glucose utilization by regulating levels of glucose-6-phosphate, an inhibitor of glucose phosphorylation.[83]

The rate of glycogenolysis and glycogen synthesis are important in regulating net hepatic glucose uptake or output. The rate limiting step in glycogen synthesis is catalyzed by uridine diphosphate glucose-glycogen transferase (glycogen synthetase).[84] This enzyme exists in two forms, the glucose-6-phosphate dependent (D) form and the glucose-6-phosphate independent (I) form[85] (Fig. 1). The interconversion of the two forms represents an important control mechanism in regulating glycogen synthesis.[86] Insulin stimulates the conversion of the D form to the I form thereby increasing the rate of glycogen synthesis and promoting hepatic glucose uptake.[87] Glycogenolysis is controlled by phosphorylase. Phosphorylase b is activated by conversion to phosphorylase a in a reaction catalyzed by phosphorylase b kinase (Fig. 1).[88] Insulin decreases hepatic phosphorylase activity and glycogenolysis by a mechanism which may be related to its effect on adenyl cyclase.[87]

A possible direct effect of insulin on the hexokinase reaction has been the subject of much speculation. Conflicting reports on the matter have done little to provide conclusive evidence in support of such a role for insulin. The nature and function of the various mammalian hexokinases have been reviewed.[89] Rat liver hexokinase exists in 4 electrophoretically distinguishable forms and one of these, hexokinase II, can be further fractionated into 2 different forms.[89] One form of hexokinase II is diminished in diabetic animals and levels of this enzyme are restored to normal by insulin.[89]

It seems likely that the metabolic effects of several hormones are mediated by adenosine 3',5'-phosphate (cyclic AMP). The biochemical effects of this nucleotide have been discussed.[90-92] Cyclic AMP is synthesized from ATP by a reaction catalyzed by adenyl cyclase and it is rapidly inactivated by a cyclic 3',5'-nucleotide phosphodiesterase which converts it to 5'-AMP.[91] Cyclic AMP levels are influenced by numerous hormones, (catecholamines, glucagon, ACTH, insulin, and others).[91] Most of these stimulate adenyl cyclase and increase cyclic AMP levels, however, insulin

decreases cyclic AMP levels by a mechanism which may involve the stimulation of phosphodiesterase.[93] A variety of substances such as methyl xanthines inhibit phosphodiesterase and increase intracellular cyclic AMP levels.[94]

It has been proposed that cyclic AMP may be a "second messenger" in hormone action in some tissues.[91] The hormone itself ("first messenger") may react with a portion of the adenyl cyclase molecule which faces the extracellular space thereby inducing conformational changes in other portions of the molecule (on the inside of the cell membrane). This could activate adenyl cyclase and increase the rate of synthesis of cyclic AMP ("second messenger") which could then produce the response.[91] This is an attractive theory and is in agreement with existing facts but more information about the chemical nature of adenyl cyclase will be required before the hypothesis can be accurately evaluated.

Some of the biochemical effects which have been attributed to increased cyclic AMP levels are: increased phosphorylase activity, decreased glycogen synthetase activity, increased gluconeogenesis, and increased lipolysis.[91]

Epinephrine and glucagon-induced glycogenolysis have been explained on this basis.[91] Both hormones activate adenyl cyclase and increase intracellular cyclic AMP levels. Cyclic AMP increases the activity of phosphorylase b kinase, thus promoting the conversion of phosphorylase b to phosphorylase a.[95,96] Cyclic AMP has also been implicated as one of the factors controlling glycogen synthetase activity. The cyclic AMP induced conversion of synthetase I to synthetase D markedly decreases the activity of the enzyme.[97] The resulting increased phosphorylase activity and decreased glycogen synthetase activity resulting from increased levels of cyclic AMP may account for increased hepatic glucose output.[98]

Cyclic AMP may also mediate the lipolytic effect of various hormones.[99] Numerous lipolytic hormones (ACTH, glucagon, catecholamines, TSH, and others) increase tissue levels of cyclic AMP by activation of adenyl cyclase.[100] This effect is potentiated by caffeine, an inhibitor of phosphodiesterase.[100] Insulin, which lowers cyclic AMP levels also inhibits epinephrine induced lipolysis.[101,102] Nicotinic acid, another lipolysis inhibitor, virtually obliterates the effects of epinephrine on cyclic AMP levels in isolated fat cells.[103] The fact that exogenous cyclic AMP stimulates lipolysis in isolated fat cells[101] adds further credence to the hypothesis that hormone induced lipolysis may be mediated by this nucleotide.

Cyclic AMP may mediate ACTH and glucagon-induced insulin release.[91] Cyclic AMP itself stimulates insulin release *in vitro*.[14] The concentration required to stimulate insulin release in these experiments was above the known physiological levels of the nucleotide but this may be due to the poor penetration of cyclic AMP into tissues.[102] Theophylline, an inhibitor of cyclic AMP degradation, stimulates insulin secretion *in vitro*[15] and *in vivo*[34] and also potentiates ACTH and glucagon-induced insulin release *in vitro*[103] and *in vivo*.[29] Other workers were unable to demonstrate a potentiation of glucagon-induced insulin release by theophylline in the isolated perfused rat pancreas[14] but this may be due to the abnormally high concentrations of glucagon employed. The proposal that insulin secretion may be mediated by cyclic AMP is consistent with the fact that insulin release is

promoted by stimulation of β-adrenergic receptors[34] and cyclic AMP is believed to mediate β-receptor effects. Further substantiation of this hypothesis is dependent on the measurement of intracellular levels of cyclic AMP in the β-cell.

The obvious importance of cyclic AMP in hormone actions indicates its importance in understanding the metabolic alterations of diabetes. The fact that only small amounts of cyclic AMP enter intact cells[104] emphasizes the need for cyclic AMP derivatives which are better able to penetrate cell membranes. Numerous studies have been carried out with N^6-2'-O-dibutyryl cyclic AMP which is a more potent activator of liver phosphorylase than cyclic AMP itself[96], probably because it enters the cells more rapidly than cyclic AMP. Other derivatives have been synthesized[105] and it is likely that further investigations by medicinal chemists will produce still other derivatives which are suitable for *in vitro* and *in vivo* studies.

Diabetes in Laboratory Animals

A tendency toward diabetes has been observed in at least 13 species and strains of animals.[106] It is anticipated that studies on diabetes in animals may provide some much needed information on the genetic aspects of diabetes, the primary metabolic lesion(s) of the disease, and the relationship between the metabolic disorders and the complications of diabetes. Much of the research in this area has been carried out with the Chinese hamster.[107-109] Diabetes in the Chinese hamster is a hereditary trait and is similar in many ways to diabetes in man. Of particular interest is the fact that vascular lesions have been observed in the diabetic hamsters.[107] Severely diabetic hamsters have a decreased ability to secrete insulin in response to glucose stimulation but mildly diabetic animals have normal plasma insulin levels. The diabetic hamster is characterized by decreased islet volume, decreased β-cell granulation, and glycogen infiltration into the islets.[110] Similar, but less pronounced changes have also been observed in non-diabetic siblings, thus indicating that certain islet cell changes occur prior to the onset of diabetes.[110] Pancreatic tissue from non-ketotic diabetic hamsters with normal circulating insulin levels secretes less insulin when incubated with glucose than does normal tissue whereas pancreatic tissue from "intermittent glycosuric" hamsters secretes more insulin than normal.[111] The sand rat also has a tendency to develop diabetes which is similar in many ways to maturity-onset diabetes in humans.[112] The initial stages of diabetes in this species are characterized by hyperglycemia and hyperinsulinemia.[113] The pathogenesis of diabetes in this species may be related to the resistance of peripheral tissue to insulin since *in vitro* experiments have shown that both muscle and adipose tissue from diabetic sand rats exhibit a marked refractoriness to insulin.[113] A tendency to develop diabetes has also been observed in the Wellesley mouse[114], the spiny mouse[115], the KK mouse[116], the Bar Harbor obese mouse[117], and numerous other species.

Numerous studies have been conducted on animals with chemically induced diabetes. Most of these studies have utilized alloxan, which is concentrated by the pancreatic β-cells[118] and decreases the number of β-granules.[119] The degree of β-cell destruction is dose dependent[120] but

the mechanism by which alloxan acts is poorly understood and the drug is rather toxic. More recently, streptozotocin[121], an antibiotic from Streptomyces achromogenes, has been used to induce diabetes in several animal species. A single dose of streptozotocin produces hyperglycemia, glycosuria, and in some cases ketonuria.[122] There is a marked depression of pancreatic insulin levels when the animals are sacrificed 3 weeks after injection.[122] It has been reported[123] that this compound induces diabetes by producing β-cell degranulation without β-cell destruction but other studies[124] make it appear more probable that it owes its diabetogenic action to a β-cytotoxic effect. The most striking evidence of β-cell necrosis is observed within 24 hours of administration of the drug[124] which could account for why some investigators have questioned its occurrence. Streptozotocin is more specific and exhibits less general toxicity than alloxan.[124] Also, the extrapancreatic lesions characteristic of diabetes are more reproducibly induced with streptozotocin.[123] It is very likely that streptozotocin will become an important research tool for studying diabetes in animals.

Oral Hypoglycemics

The introduction of the first oral hypoglycemics in the 1950s was an important event in the history of the treatment of diabetes. Five such compounds are currently available. Their therapeutic usefulness is well established and their metabolic effects are well documented but there are still a number of questions to be answered in regard to their mechanism(s) of action.

Sulfonylureas. The current status of tolbutamide in particular and the sulfonylureas in general, was the subject of a recent symposium.[125] It is generally believed that these compounds owe their activity to their ability to stimulate insulin secretion, thus accounting for their ineffectiveness in treating juvenile diabetics or other diabetics with no endogenous supply of insulin. The mechanism by which they promote insulin release is not clearly understood although it is quite certainly different from the mechanism by which glucose stimulates insulin release.[126] This explains why many diabetics can secrete insulin in response to sulfonylurea stimulation although they have lost the ability to respond to a glycemic stimulus. Numerous attempts have been made to implicate extrapancreatic effects in the mechanism of action of sulfonylureas but the evidence in support of such a case is less than convincing.[127,128] Sulfonylureas inhibit lipolysis and block the effects of various lipolytic agents in isolated fat cells[129] and also suppress ketogenesis in rat liver slices.[130] These effects can be produced in the absence of glucose and insulin and probably represent direct effects of the sulfonylureas, but there is no evidence that these effects are releated to the hypoglycemic activity of the sulfonylureas. It has recently been shown that patients undergoing long-term therapy with chlorpropamide demonstrate an improvement in glucose tolerance which is not accompanied by increased levels of circulating insulin.[131] The significance of these findings is unclear in view of the fact that the study of insulin release in in vivo systems is very complex and the results of such studies are often difficult to interpret. It

might also be pointed out that it is not circulating insulin, but rather insulin in contact with the cell membrane or inside the cell, which promotes glucose utilization. These results are, however, in agreement with results from in vitro experiments which show that tolbutamide-induced insulin release decreases with time, possibly because of intracellular feedback inhibition.[132] Clinical studies with glyhexamide, a new sulfonylurea hypoglycemic have been reported.[133] The results indicate that this compound is comparable in potency to tolbutamide but is less potent than chlorpropamide and probably has no particular advantages over the currently available sulfonylureas.

Biguanides. The metabolic effects of the biguanides were discussed in detail in last year's review and are the subject of a recent paper.[134] The theory that the biguanides exert their primary effect by interfering with electron transport and oxidative phosphorylation gains additional support from the finding that there is a correlation between phenformin inhibition of pyruvate oxidation and its effects on glucose metabolism. Reports of initial weight loss in association with phenformin therapy continue to appear[135] but the reason for this weight loss is still a debatable issue. The mode of action of phenformin, its effects on glucose tolerance, plasma insulin, serum lipids, and body weight were discussed at a recent symposium.[136]

Other Compounds. Recent studies on the metabolic effects of 5-methylpyrazole-3-carboxylic acid indicate that it exerts its primary effect on adipose tissue where it inhibits lipolysis, increases the incorporation of glucose into glycerides, and promotes glycogen synthesis from glucose.[137] It does not stimulate the incorporation of glucose into muscle glycogen in vitro. These findings are in agreement with earlier reports which suggested that its hypoglycemic activity is secondary to its effect on plasma FFA levels.[138] The observation that rats develop resistance to this agent[139] and also to 3-methylisoxazole-5-carboxylic acid[140] is unfortunate and decreases the likelihood that these agents will be clinically useful. The hypoglycemic activity and the metabolic effects of pyrazinamide have been examined.[141] It decreases blood sugar in intact, adrenalectomized, hypophysectomized, and eviscerated rats. Its pharmacological action seems to be similar to that of the pyrazoles and isoxazoles in that its hypoglycemic effect appears to be secondary to its effect on plasma FFA. Recent experiments indicate that tecomine and tecostamine, alkaloids from the leaves of Tecoma stans, lower blood sugar in fasted and alloxan-diabetic rabbits but not in pancreatectomized rabbits.[142] Although no detailed mechanistic studies have been reported, it appears that their action is dependent on the presence of a functioning pancreas so it is unlikely that they offer any important advantages over existing compounds. 5-Methoxyindole-2-carboxylic acid lowers blood sugar in the alloxan-diabetic mouse by a mechanism which may be involved with inhibition of hepatic gluconeogenesis.[143]

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Chapter 17. Atherosclerosis

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Introduction - The general pattern of research in atherosclerosis needs a sharpening of the focus on that part of the vascular bed that seems to deal the most critical blow to maintenance of life when it becomes either inoperable in toto for a short period of time or is reduced in function for a more extended period of time. "It is now axiomatic that sclerotic changes in the coronary arteries are at the root of 90% of all genuine forms of angina pectoris and myocardial infarction."[1] Whenever possible, I hope to direct attention to coronary atherosclerosis. To more clearly define research objectives, to more precisely control the methodology and more crisply scrutinize the data and the conclusions drawn therefrom, the literature should be lucid in the differentiation among coronary atherosclerosis, aortic atherosclerosis, cerebral atherosclerosis, and atherosclerosis of any other reasonably definable part of the circulation system. Hereditary factors, including obesity, hypertension, "inborn errors" of hyperlipidemia, diabetes, deranged thyroid function, malfunction of the adrenals and many others, have been implicated in the development of atherosclerosis. Environmental factors may be of primary or secondary influence; - - these include emotional stress situations, diet, climate, detrimental habits (smoking, lack of exercise, etc.) contracted diseases, certain drugs - - many of these too, cause hypertension, obesity, diabetes, and other metabolic disorders. At present, no single factor can be isolated as exclusive in the etiology of coronary atherosclerosis. Furthermore, no one simple definition of atherosclerosis, even though the word is used universally, carries a unanimous stamp of approval among the multitude of researchers studying the disease.

I must emphasize the importance of studies of coronary atherosclerosis rather than just aortic atherosclerosis. First, the coronary arteries are anatomically different from the aorta, and consequently have a different overall metabolic behavior. Secondly, arteries from the several parts of the cardiovascular system are subject to a spectrum of dynamic forces from inside and outside the vessel walls which produce different modes of nutrition in the walls of the various kinds of arteries. Metabolic nutrition, including provision of oxygen to the coronary muscle itself, depends on the integrity of not only the large coronary arteries but to a large extent on the small vessels within the muscle. Whenever the delivery of blood with its oxygen and nutrients is decreased in the heart, further problems develop within the heart muscle, eventually producing diminished performance and finally failures.

Coronary arteries afflicted with "atherosclerosis" show thickening of the arterial intima, and usually hypertrophy of the media, with clearly demonstrable lipid deposits (including cholesterol) within the intima or subintima. All of this tends to reduce the size of the arterial lumen and restrict the flow of blood at reasonable pressures. Research must

endeavor:

1. to elucidate the cause(s) of coronary atherosclerosis and its morbid sequalae,
2. to discover ways (including drugs) to prevent, attenuate and, hopefully, cure the disasterous disease, and, finally,
3. to apply the knowledge prophylactically and therapeutically to achieve productive longevity of the human race.

Advances in these areas are of primary importance to medicine and medicinal chemistry. What has been the progress in the areas of concern in 1967?

Etiology - In the study of the causes of atherosclerosis, the factors found most likely to be present in the living system prior to death, in relation to the proven presence of coronary atherosclerosis at autopsy, are advanced age, elevated serum cholesterol, and elevated blood pressure, with elevated blood lipids and the presence of diabetes appearing influential. Other measurable metabolic parameters found to correlate as coronary heart disease risk factors are elevated serum uric acid[2,3], lowered serum albumin levels,[4] lowered lipoprotein lipase[5] and lowered endogenous heparin.[6] Of these factors, "cholesterol" continues as a favorite research topic.

Reviews of the literature[7,8] reveal numerous studies on atherosclerosis are concerned with cholesterol, its presence in or absence from the diet, its synthesis and catabolism, and its distribution among plasma and various tissues. Epidemiological studies in man suggest that diet - high in fat or cholesterol along with fat - plays a prominent role in the human atherosclerotic disease. Cholesterol, linked to the disease by its presence in the atherosclerotic plaques and its effect in diets of animals, is derived both from diet and by biosynthesis in body tissues. Rats and rabbits, so often used in laboratory studies, differ greatly in susceptibility to atherosclerosis and may well be quite different from humans in this respect. Even on semi-synthetic diets, containing no added cholesterol, rabbits develop atherosclerosis, whereas they do not on stock diets.[9] Furthermore, the plasma cholesterol of rabbits is more easily altered than that of rats, whereas the cholesterol synthesis rate (from acetate) varies widely in rats in response to various diets.

Studies in which dietary factors are manipulated, especially in animals, to produce "atherosclerosis", are numerous and studies to produce variations in serum cholesterol levels are even more plentiful. Rabbits still hold the spotlight in dietary studies involving cholesterol feeding with or without added fats, saturated or unsaturated. Without cholesterol added, few atheroma are found.[10] However, saturated fats appear more atherogenic than unsaturated fats when fed along with cholesterol.[11] Heated corn oil in the diet was more atherogenic than unheated oil.[12] After 12 weeks on an atherogenic diet followed by 12 weeks of regular or semi-starvation diet, rabbits were found to have an intensification of atherosclerosis (increase in aorta plaque intensity of 36 $\pm$ 5.9% and 58.8 $\pm$ 7.6%, respectively, for the two final diets).[13] Rabbits receiving corn oil and coconut oil diets (no added cholesterol) alternately, or as a 50-50 mixture, for 10, 10 week periods, developed more aortic

and coronary atherosclerotic lesions on the alternate regimen than on the 50-50 mixture.[14] Injection of lipid peroxides into abdominal aortas of rabbits did not elicit plaque formation at the injection site, even though evidence of presence of peroxides in tissues in vitro is highly suggestive that the peroxidation goes on in vivo.[15]

Studies designed to demonstrate directly the causal relationships of various "prime factors" of coronary atherosclerosis in man and animals are few indeed. Recently, studies were reported in which a rat, usually considered resistant to atherosclerosis, was used as a model for coronary atherosclerosis study.[16] The factor of hypertension was added to the study as well as a longer period of study time. These renal hypertensive rats developed coronary artery lesions having some of the characteristics of those in man. Rats made hypertensive by DOCA implantation did not develop lesions. Dietary salt loading increased the incidence and severity of the coronary lesions slightly in DOCA hypertensive rats; the severity, even though already high, increased somewhat among the renal hypertensive rats with added salt.

Human cholesterol metabolism has been extensively reviewed recently[17] and the lack of negative "feed-back" control was concluded. Cholesterol rich diets (greater than 2.5 - 3 gm/day) had a rather marked influence on human serum cholesterol - but not on liver biosynthesis. However, man's principal protection from high oral intake of cholesterol is the limited capacity for intestinal absorption. Serum cholesterol of young male humans on a butter-containing diet was increased significantly over that of others receiving corn oil margarine.[18] Kritchevsky reviewed experimental atherosclerosis in Primates and other species[19] and pointed out that a number of animal species might serve as animal models for study of certain aspects of the disease. Among these are rabbits on a semi-synthetic diet, squirrel monkeys on high fat diets, and rhesus monkeys and baboons on atherogenic regimens. These latter two animals are somewhat large, and, like pigs, tend to resemble man more closely than birds and rabbits. However, no absolute and uniform relationship between dietary factors and development of naturally occurring atherosclerosis in a number of animal species has been found.

Prophylaxis - Since diet seems to influence serum cholesterol levels and be involved in production of atherosclerosis, certain changes might prevent the development of the disease. Jones[20] reviewed the effect of diet on the "Pathogenesis of Coronary Disease" and states: "We have no assurance as yet that any dietary manipulation aimed at reducing serum cholesterol will benefit the course of the disease." Still, coupling diet with other conditions, and the inclusion of hypocholesterolemic drugs or preparations from natural sources, have become popular research maneuvers. Rabbits on an alfalfa diet plus cholesterol did not get hypercholesterolemia nor atherosclerosis;[21] pre-existing atherosclerotic lesions were not affected by alfalfa feeding. Lipid-laden atherosclerotic coronary artery lesions were induced in rats by X-irradiation (600r) plus a cholesterol containing diet (1%).[22] Chondroitin sulfate A (at 0.4%) in the diet markedly reduced the incidence and severity of coronary lesions;

subcutaneous injections of the material were without effect.[22] Linoleic acid,[23] or its ethyl ester,[24,25] in cholesterol containing diets, prevented or suppressed the development of atherosclerosis in various animals. On the other hand, N-cyclohexyl-linoleamide, while showing a good cholesterol lowering effect in cholesterol fed rabbits,[26,27] suppressed aortic atheromata only at high doses (600 mg/day);[26] N-(a-methylbenzyl-linoleamide was more effective both ways.[27] In another study, N-cyclohexyl-linoleamide, orally, in a cholesterol diet of rabbits, decreased plasma, serum, and liver cholesterol below control values and inhibited atheroma development.[28] But, it was reported also in this study that feeding of linoleic acid, ethyl linoleate and safflower oil did not affect serum cholesterol or atherosclerosis in rabbits.

Cholestyramine, an insoluble, bile acid binding polymer, was effective in preventing the accumulation of tissue cholesterol in rats fed atherogenic diets.[29] Cholestyramine also was found to prevent formation of gallstones in hamsters on a cholesterol gallstone inducing diet - it likewise brought about dissolution of already formed stones.[30] (In previous studies cholestyramine was found not only to bind bile acids but also thyroxine.)[31] Pectin included in cholesterol (1%) diets of rabbits caused lower blood cholesterol and less aortic atherosclerosis but no difference in the incidence and severity of coronary lesions.[32] Powdered mushroom (or an isolate) was found effective in preventing a rise in serum cholesterol of cholesterol fed rats.[33] Orally administered Dextran and cellulose anion exchangers were hypolipidemic in cockerels and dogs,[34] probably due to bile acid sequestration. Dextran, (6%, 30 ml/day), given intravenously to cholesterol-fed rabbits, reduced the rise in serum cholesterol, phospholipid, and triglycerides, apparently due to expansion of plasma volume.[35] Cessation of dextran permitted large increases in the serum lipids. On the other hand, intravenous low-molecular weight Dextran had no effect on rat and human serum cholesterol.[36]

Renese (0.001g) or Hygroton (0.050g.) every 2nd day in a cholesterol diet (.2g chol/kg) of rabbits for 3 months prevented atherosclerosis in aorta, coronary, and pulmonary arteries; controls were atherosclerotic.[37] Daily oral administration of Dilantin to rabbits on a regular diet for 30 days reduced aortic cholesterol and phospholipids and increased triglycerides with no change in liver and plasma values. However, Dilantin together with an atherogenic diet (up to 135 days) resulted in an acceleration in the development of aortic atherosclerosis.[38] Pyridinolcarbamate (orally, 20 mg/Kg/day) has been reported ineffective in reducing the serum cholesterol, aortic atherosclerosis or coronary atherosclerosis in monkeys fed a butter and cholesterol diet.[39] (This compound was previously found effective in humans.) Norethynodrel (2mg/day, orally) was hypocholesterolemic in cholesterol fed rabbits and reduced somewhat the aortic atherosclerosis development.[40] Cholestane-triol and its derivatives (orally) were found to lower blood and liver cholesterol and atherogenesis in cholesterol fed rabbits; in chickens, serum and liver cholesterol were lowered; whereas, in rats, only the liver cholesterol was lowered.[41,42] Prednisone (orally, 15 mg/day, 5 days/week) was found to diminish the rise in serum, adrenal, and liver cholesterol and aorta atherosclerosis in

rabbits on atherogenic diets.[43] Dehydroepiandrosterone, (5 mg/Kg/day orally for 10 days) was found antihypercholesterolemic in rats with elevated plasma cholesterol, due to propylthiouracil and cholesterol feeding.[44]

In renal hypertensive rats that would normally develop hypercholesterolemia and coronary atherosclerosis on low cholesterol diets, propylthiouracil (0.8 - 4.0 mg/rat/day, orally) permitted hypercholesterolemia, but prevented the development of coronary atherosclerosis in a 30 week study;[45] no aortic atherosclerosis was found. Another investigator in a slightly different study found that renal hypertensive rats on 40% egg yoke diet with 0.3% propylthiouracil, developed both coronary and aortic atherosclerosis.[46]

Nicotinic, nicotinuric, or 3-pyridineacetic acid (100 or 250 mg/kg), given intraperitoneally to rats, counteracted the hyperlipidemic and hypercholesterolemic effect of intraperitoneal tyloxapol (200 mg/kg); nicotinuric acid had the greatest effect.[47] In rats receiving an atherogenic diet, sodium nicotinate did not prevent lesions in the cardiovascular system; sodium 3-pyridylacetate potentiated the atherosclerotic changes while sodium nicotinurate inhibited the pathologic changes.[48] Biotin (400 µg daily, 6 months), administered subcutaneously to rabbits on a cholesterol diet, prevented the development of aortic atherosclerosis as well as decreased plasma and liver cholesterol levels and β-lipoprotein in blood.[49] Hepatocatalase peroxidase (beef liver isolate, intramuscularly, 5 mg/day, for 15 weeks) reduced cholesterol induced hypercholesterolemia and atherosclerosis in rabbits, with no apparent toxicity or side effects.[50] PS-179, a protein anabolic steroid, given (0.3 - 3 mg, subcutaneously, daily) to rabbits receiving a synthetic, low protein, cholesterol (1%) diet inhibited aortic atherosclerosis, lowered tissue lipid deposits, was hypocholesterolemic, and improved the NEFA plasma composition; it reduced slightly the incidence of coronary atherosclerosis.[51] Methyltestosterone was completely without beneficial effect.[51]

Thus, it appears there are several agents that can prevent atherosclerosis in animals - for the most part in aortas of animals that are abnormally loaded dietarily with extra cholesterol and/or added fat. Even though some evidence suggests so, it still is not clear that a low cholesterol, low fat diet will prevent atherosclerosis development in humans. (Diet modification studies on a large scale in adults, in an attempt to stop or reverse the disease, have been in progress for some time; reports are due in 1968.) The inclusion in human diets of agents that are effective in animals still needs long term investigations for preventive evaluation.

Therapy - The clear-cut identity of the presence of arterial disease and an irrefutable demonstration of its disappearance, constitute the basis for judgement of a therapeutic effect. At present, absolute knowledge of the in vivo presence or cure of atherosclerotic lesions, especially in human coronary arteries, is difficult to obtain. Suspicion of a therapeutic effect may be established on the basis of long term follow-up of treated patients with clinically "established" symptomatic disease,

especially if they survive and the symptoms disappear.

Cholesterol has been implicated in the etiology of atherosclerosis in animals and an elevated serum cholesterol, in both laboratory investigations and epidemiological studies, appears indicative in man. Therefore, normalization of serum cholesterol, though not necessarily therapeutic in itself, might indicate - or at least suggest - a step in the right direction, provided there is parallel evidence that cholesterol or other deleterious substances are not being pathologically deposited in tissues. Furthermore, beneficial modification of lipid metabolism in general would appear to have some logical place in a therapeutic regimen.

Atromid, a combination of clofibrate and androsterone, was found to be effective in humans for lowering serum cholesterol, triglyceride, and phospholipid levels,[52] cholesterol and total lipids,[53] (but not platelet stickiness),[54] and cholesterol, phospholipid and beta-lipoproteins.[55] On the other hand, in another report, Atromid decreased blood cholesterol, caused a slight increase in phospholipid, lowered beta-lipoprotein, and appeared to reduce the incidence and severity of angina pectoris attacks, but, had no effect on triglyceride.[56] The effectiveness of clofibrate by itself, without androsterone, on serum cholesterol has been recorded in several reports[57,58] with no significant effect on human bile acid patterns;[59] no effect on plasma fibrinogen (in contrast to previous report of 1966),[60] nor much fibrinolylic activity,[61] but some effect on euglobulin lysis time (a measure of plasminogen activator).[61] Clofibrate[62] was found not to affect globulin-bound thyroxine but cleaved prealbumin- and albumin-bound thyroxine, possibly increasing free thyroxine in liver. (Such a possibility was suggested several years ago.) In a study of patients without exceedingly elevated serum cholesterol level, clofibrate caused no significant change in serum cholesterol or phospholipid but did decrease serum triglyceride.[63] Clofibrate has not been used long enough to establish its effectiveness in prolonging life of coronary atherosclerotic patients or its effectiveness in removing suspected atheroma from affected arteries even though it has been reported effective in xanthomatosis.[62] Reports differ on the "absolute" effectiveness of clofibrate on the various lipid parameters. However, it does appear to be effective in many studies and possibly variations in experimental procedures and subjects account for the lack of uniform results among investigators.

Other compounds that have been reported to have hypocholesterolemic properties in humans include: Metrazol,[64] D-thyroxine,[65] erythritol plus tetranicotinate,[66] 3-hydroxy-3-methylglutaric acid,[67] aminazine,[68] neomycin,[69-72] phenindione;[73] 17-(3-hydroxy-1-propynyl)-3-methoxyestra-1,3,5,(10)-trien-17B-ol,17α-hydrocinnamate,[74] Fradiomycin.[75] Metamucil,[76] an oral hydrophilic colloid, and cholestyramine[77,78] lowered serum cholesterol and increased bile acid excretion. Several series of compounds with members having serum cholesterol lowering possibilities have been prepared.[79-87] Further investigation of several of the individual compounds may show whether or not they are effective against atherosclerosis.

Thrombosis prevention and fibrinolysis may be involved in prophylaxis and therapy in atherosclerosis. Even though there seems to be a

relationship between serum lipid variations and platelet adhesiveness changes in atherosclerotic subjects, no "cause and effect" has yet been proven.[88] Clofibrate or Atromid had no effect on the coagulation processes in elderly women[89] nor did these two agents affect platelet adhesiveness in multiple sclerosis patients.[90] Injection of fibrinolysin daily for 100 days into cholesterol fed rabbits did not alter hypercholesterolemia but appeared to have a slight retarding effect on aortic lipoidosis.[91] The fibrinogen level of the blood was reduced by cholesterol feeding.[91] Long-term anticoagulant therapy was reviewed by Jones[20] and even though an"imperfect benefit" was found, it did appear to be worthwhile "in patients under 60, for at least two years."

If rabbits on atherogenic diet (2% cholesterol in 6% corn oil) for 8 weeks are returned to normal diets for 8 weeks, their atheroma became more severe.[92] D-thyroxine (0.50 mg/day) or L-thyroxine (0.05 mg/day) given by gavage during the post-cholesterol feeding period have no effect on the progression of pre-established lesions, but, if the drugs are given in the food, they reduce the severity of the atheromas as compared to untreated controls. Furthermore, if the drugs are given during the cholesterol feeding period in the two fashions above, the atheroma are comparably less. Apparently, the atheroma do not follow the levels of serum cholesterol since cessation of cholesterol feeding produces a sharp fall in hypercholesterolemia. It has been reported in another study[93] that atheroma induced in rabbits by cholesterol diets of short duration may be reversed but lesions of long standing may be resistant to reversal. At the moment, there is no "sure" therapy for atherosclerosis -- aortic or coronary! But, if lowering serum cholesterol is part of the therapeutic measures, there are some leads.

Miscellaneous - Studies of cholesterol metabolism have moved away from biosynthesis inhibition in liver. It has been found that cholesterol synthesis in livers of cholesterol fed or fasted rats was markedly below that of controls, whereas synthesis in other tissues was relatively unaffected.[94] The highest rate was found in the gastrointestinal tract. However, the liver, as a "whole organ", still has the potential for the greatest total amount of cholesterol synthesis. In regulation of cholesterol synthesis, it appears that extra-hepatic processes contribute substantially to maintenance of cholesterol "balance." One such study was conducted using rats and cholestyramine and/or tri-iodothyropropionic acid.[95] It was concluded that, "control of cholesterol metabolism in the intact animal does not reside at any one site and may not reside in the liver."

Few studies have been designed to elucidate and contrast the normal metabolic processes of coronary arteries and the atherosclerotic processes. Basic studies of xanthomas may reveal metabolic processes applicable to atherosclerosis investigation.[96] Apparently, there is a relationship between circulating serum lipoprotein levels and the size of the xanthomas,[97] thus a relation to deposition and removal of xanthoma material.

Arterial wall metabolism depends on the functioning structure of the

vessel wall and surrounding tissue. The "fine structure" of both normal and atherosclerotic arteries - - aortic and coronary - - has been compared in a recent review.[98] Abnormal numbers of smooth muscle cells in subendothelial spaces of the arteries may be intimately involved in synthesizing and accumulating lipid and developing atherosclerosis. Why the muscle cells appear in this area and by what route have not been delineated clearly yet! A detailed study of human aortic and coronary arterial material[6] revealed the prevalent localization of heparin like substances in the superficial intima area and of chondroitin sulphates and sialic acid substances in the deep intimal area. Furthermore, it was found that the coronary intima had more enzymatic activity than the aortic intima, whereas the sialic acid concentration was higher in the aortic intima. Thoracic aortas of atherosclerotic men (50-60 years of age) had a low proportion of heparin sulphate both in the superficial and deep regions of the atherosclerotic plaque;[99] the sialic acid/uronic acid ratio of the superficial intimal zone was low, but in the deeper fatty zone of the plaque, it was high. Hence, it appears that atherosclerosis is favored by a decrease in the heparin-like activity and by an increase in the chondroitinsulphate and sialic acid in the tissues.

Recent studies of vascular enzymes have been reviewed by Zemplenyi.[100] The available data indicate that aortic enzyme activities tend to decrease over the age of 40 in humans, although lactate dehydrogenase and β-glucuronidase are exceptions. Some enzymes are sex linked, and venous tissue exhibits greater activity of most enzymes than arterial tissue. Different segments of the the arterial tree have different enzyme patterns - some segments are more susceptible to atherosclerosis than others. Enzyme activity of an arterial segment is clearly a consequence of the smooth muscle cell content and condition. Their metabolic activity appears to decline following vascular injury.

Thus, in arterial segments susceptible to atherosclerosis, in which the activity of Krebs cycle enzymes decreases, there is a decrease of energy-rich phosphate bonds and reduced vascular synthetic activity for producing proteins, including enzymes. Consequently, defense mechanisms to protect the wall from sclerogenic materials are reduced and further injury occurs, producing atherosclerosis. No conclusive data to support the idea of "improper balance" between disturbed plasma lipids and protective mechanisms of the arterial wall are presently at hand.

Summary and Comment - In 1967, the general field of atherosclerosis research has moved gently forward. Studies of cholesterol and its management still consume much research effort. The licensing of clofibrate for use in the United States and design of cholesterol lowering diets have stimulated the hope of an answer to, "Will management of cholesterol really lower disability and death due to cardiovascular disease and atherosclerosis?" Data on the effectiveness of several drugs now being studied in a large scale national program will be collected over the next several years. Even though many segments of the atherosclerosis problem are under intensive investigation, the specific areas of continued research

should primarily include:

1. Continuation of the search for specific etiologic factors to identify and to inter-relate them;
2. Development of a laboratory animal model that gets coronary atherosclerosis lesions comparable to those of the human and in a manner similar to man;
3. Identification of the specific points and times, along the disease progress path, at which the disease process can be interrupted with minimum danger (maximum safety) to the patient as far as altering the patients'metabolic patterns - - preferably, back to normal!

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Chapter 18. Non-Steroidal Hormones and Their Antagonists

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Introduction.-Recent advances in non-steroidal hormone research are dominated by structure elucidation and synthesis of protein and polypeptide hormones and the characterization of a family of hormone-like lipids. The latter, known as the prostaglandins, represent an exciting and important group of compounds which deserve consideration under the title of this chapter. However, because the prostaglandins have been the subject of a number of very recent reviews[1-4] and because there is such a wealth of new information on the polypeptide hormones, the authors have chosen to omit detailed consideration of the lipid hormones in this review. The development of radioimmunoassays[5] which permit detection of polypeptides and proteins in the ng range, has contributed significantly to progress in this field, but detailed consideration of this development also lies outside the scope of this endeavor. Indeed, even within the scope of the subject matter covered, space limitations have necessitated a considerable amount of selection. Therefore, what follows is not a comprehensive review but rather a summary of what the authors regard as the most significant recent achievements which further our understanding of the "glandular hormones."

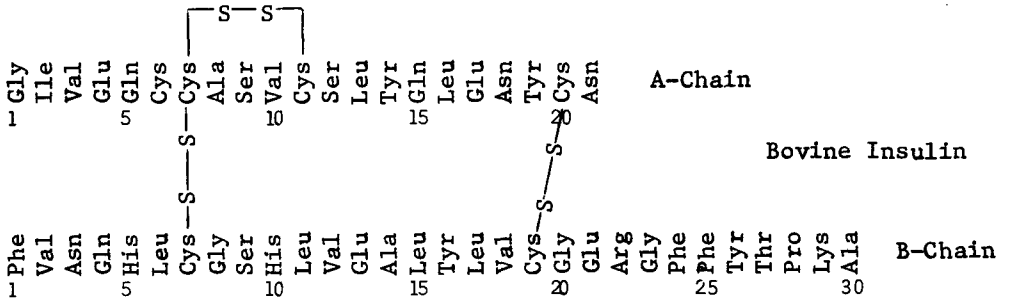
HORMONES OF THE PANCREAS

Insulin.-Since reviewed by Jorgensen[6] Zahn[7] summarized the synthetic work as of early 1966 and pointed out that new methods must be developed to permit synthesis of insulin on a commercial scale. A preliminary report[8] from the Aachen group of an improved B-chain synthesis involving symmetrical cystine peptides appeared recently. Kung et al[9] reported isolating µg quantities of crystalline insulin from combination mixtures of synthetic and natural chains and of all-synthetic chains of bovine insulin. Significant, however, is the fact that in this work the overall yield of isolated synthetic crystalline insulin was only 0.1% of that theoretically predicted. Similarly, Marglin and Merrifield[10] reported the synthesis of bovine insulin by the solid phase method, but the yield of isolated product was extremely small and of low specific activity.

The approach to a practical total synthesis requires careful study of the three main problems involved, namely, synthesis of the A- and B- chains in the required state of purity and optical integrity, combination conditions which provide reasonable yields, and development of techniques for isolation and crystallization of the insulin formed. All three of these problems have been systematically investigated by Katsoyannis[11] and his group, and significant progress was made in each area. A new method utilizing the S-sulfonated derivative of the B-chain reacting directly with an excess of the sulfhydryl form of the A-chain provided 50-65% overall yields of recombined insulin.[12] Using this method the yield of insulin from totally synthetic A- and B-chains was dramatically increased. The combination yields of the half-synthetic insulins ranged from 17-42% and for the all-synthetic insulins, 12-22%.[13] Improvements in the procedures for isolating insulin

from combination mixtures permitted recoveries in the range of 35-52%.[13] The specific activity of the various insulin hydrochlorides obtained ranged from 22 to 25 IU/mg vs. 23-25 IU/mg for the natural insulin. Crystallization was complicated by many factors, but at least in some cases, e.g., all-synthetic sheep, a crystalline form similar to that of the natural hormone was obtained.

Until recently it was thought that species differences in amino acid sequence were quite limited and that most of the variations which did occur were confined to positions 8, 9 and 10 of the A-chain. Now that the sequence has been determined for insulins of a larger number of species this idea has been dispelled. According to Smith,[14] residues have been replaced in at least 29 of 51 positions and small variations in chain length have been discovered. Although multiple insulins have been reported for fishes,[14,15] the rat is the only mammal that has so far been shown to produce more than one form of insulin.[14] Guinea pig insulin has 17 alterations compared to pig insulin.[14] This is a large variation considering the relative constancy found among other mammalian species. In spite of these differences, there appears to be little variation in the biological activity of these insulins as measured by standard mouse convulsion and blood glucose assays.[15-17]



Carpenter[18] has selectively modified portions of the insulin molecule by enzymatic and chemical means and determined the effect on biological activity. His findings suggest that the C-terminal Asp or Asn of the A-chain is necessary for full expression of biological activity and that in the B-chain some part of the heptapeptide sequence C-terminal to the Arg at 22 is required. The latter finding is supported by Bromer and Chance[19] who found their highly purified desoctapeptide-insulin retained less than 1% of the biologic activity and about 1% of the immunologic reactivity of native insulin. Reduced activity of insulin derivatives in which the 3 free amino groups have been substituted indicates that they are important and may be needed for proper binding of the hormone to its receptor site. Acetylation[20] of these groups diminished activity by 25%, whereas addition of 3 DNP groups[21] resulted in complete loss, and t-BOC groups[22] reduced activity to ca 20% of that of the native hormone. Recently, Levy and Carpenter[22] have modified bovine insulin by the addition of triAla-, triAsp-, triLys-, triMet-, and triGlu- to the 3 free amino groups. Despite the large differences in size, charge and hydrophilic-hydrophobic proper-

ties of the groups introduced, the resulting triaminoacyl-insulins all possessed 40-50% of the activity of bovine insulin as measured in the mouse convulsion test and slightly higher values in immunochemical assays. Deamidation of commercial insulin on storage or acid treatment yields predominantly the desamido-insulin with a C-terminal Asp rather than Asn.[18,23,24] Berson and Yalow[25] suggest that in cases of antigenicity of porcine insulin in man it is possible that the desamido compounds might represent the antigenic components. The possibility of a circulating insulin antagonist has been considered in the etiology of diabetes mellitus.[26] Although controversy exists over the nature and demonstrability of the factors proposed,[27-29] it is interesting that a complex of reduced insulin B-chain and albumin has been reported to be antagonistic to insulin action both *in vitro*[30] and *in vivo*.[31-33] Finally, sulfated bovine insulin[34] has been reported to be a promising advance in the treatment of insulin-resistant diabetes.[35]

Glucagon.-According to Sokal,[36] glucagon is secreted by the α-cells of the pancreas in response to hypoglycemia or starvation and acts as a mediator of the normal hepatic response to these states. He considers it quite possible that deficient glucagon secretion contributes to "reactive hypoglycemia" and to the hypoglycemic episodes of "brittle" diabetes, and that hypersecretion may play a role in maturity-onset diabetes. The availability of a sensitive radioimmunoassay[37] is helping to elucidate more clearly the role of glucagon in mammalian physiology and metabolism.

Porcine Glucagon

His	Ser	Gln	Gly	Thr	Phe	Thr	Ser	Asp	Tyr	Ser	Lys	Tyr	Leu	Asp	Ser	Arg	Arg	Ala	Gln	Asp	Phe	Val	Gln	Try	Leu	Met	Asn	Thr
1				5					10					15					20					25				29

Work on the synthesis of the nonacosapeptide sequence proposed by the Lilly group in 1957 has continued in several laboratories. New partial sequences reported include 17-19,[38] 13-16,[34] 16-29,[40] and 22-29.[41] The first total synthesis of the hormone was reported by Wünsch.[42] The synthetic nonacosapeptide was shown to be chromatographically and electrophoretically identical to the natural bovine hormone but it possessed only 50% of the biological activity. An interesting structural similarity between insulin and glucagon was reported by Schuster.[43]

HORMONES OF THE PITUITARY

Growth Hormone.-In contrast to most of the hormones of the anterior pituitary, growth hormone (GH) or somatotropin does not appear to have a specific target organ, but does possess important metabolic regulatory activities. Classically GH is required for normal growth and long-bone development.[44] Its precise role in the adult mammal, including man, remains obscure. Chemical, biological and clinical studies with highly purified preparations of bovine (B), ovine, porcine and human (H) GH have clearly established the individuality of the hormone for each species and the lack of interspecies activity in most cases.[44,45] A notable exception is the rat which can utilize GH from many mammalian species. In man only GH from primate species is active. GH from a variety of species has been subjected

to limited enzymatic digestion and the modified hormone retained significant activity as measured in the rat.[46] These facts led to speculation that there is a common active "core" in GHs of mammalian species and that the rat has the capability of degrading non-rat GH to liberate this core, but higher species do not. Attempts to selectively modify BGH to render it active in man have offered some encouragement for this hypothesis;[47-49] but the final answer requires systematic study of structure-activity relationships. A most important step toward realization of this goal was the elucidation of the major part of the 188-amino acid sequence of HGH by Li, Liu and Dixon.[50] Significant advances to knowledge of the chemistry of BGH have been made by the Buenos Aires group, first by showing that in contrast to previous reports the true mol. wt. is ca 20,800[51] (almost identical to that of HGH rather than double) and recently by proposing a partial sequence.[52] In Fig. 1 the sequence of HGH[50] is indicated with homologous fragments of BGH placed alongside. Solid lines indicate identical sequences; broken lines are used when the sequence in BGH is not known but homology with the corresponding fragment in HGH is assumed from the amino acid composition. In cases where an amino acid in HGH is replaced by a different one in BGH, the replacement is indicated in the BGH line. Deletions are indicated by X and unknown sequences are enclosed in parentheses. Considerable analogy exists between the two structures with regard to similar location of the disulfide bridges and similar sequences C-terminad of position 80. The N-terminal area of BGH (not shown) has a sequence only vaguely related to HGH.

Adrenocorticotropin (ACTH).-Synthetic 1-24 ACTH has the first 24 amino acids of natural β-1-39 ACTH and possesses full activity. D-Ser^1-Nle^4-(Val-NH$_2$)^{25} 1-25 ACTH,[53] was more active in dexamethasone-blocked humans than 1-24 ACTH. D-Ser was substituted for L-Ser at the N-terminus to resist degradation by carboxypeptidases, and Met^4 was replaced by Nle because of the inactivation following methionine-sulfoxide formation.[54] D-Ser^1-17,18-diOrn 1-24 ACTH was compared with 1-24 ACTH and found to be 5x more active in man.[55] To determine whether the tetrapeptide sequence Lys-Pro-Val-Gly at positions 11-14 in synthetic 1-19 ACTH is necessary for adrenal- and melanocyte-stimulating activities only as a bridge or spacer between the N-terminal decapeptide and the basic core at 15-18, positions 11-13 were replaced by Gly in the following peptides: Gly^{11,12,13} (1-19) ACTH, Gly^{12,13} (1-17) ACTH amide, Gly^{11,13} (1-17) ACTH amide and Gly^{13} (1-17) ACTH amide.[56] As a group these peptides exhibited much less ACTH activity, especially the Gly^{13} compound, illustrating the importance of Val^{13} for full steroidogenic potency. Therefore the effect of the Lys-Pro-Val sequence is specific for full ACTH and MSH activity, and not that of a nonspecific spacer. The lack of essentiality of the imidazole-histidine was shown by synthesizing 5-Gln, 6-β-(pyrazolyl-3)-Ala-1-20 ACTH amide,[57] which had 60% of the standard activity. The lack of effect from change in acid-base characteristics may be due to preservation of peptide conformation since both imidazole and pyrazole are isosteric aromatic ring systems.[58] Mice treated with synthetic 1-24 ACTH had increased numbers of ovarian follicles, renal glomerular lesions, adrenal stimulation, and decreased thymus weight (the effects of the natural hormone).[59]

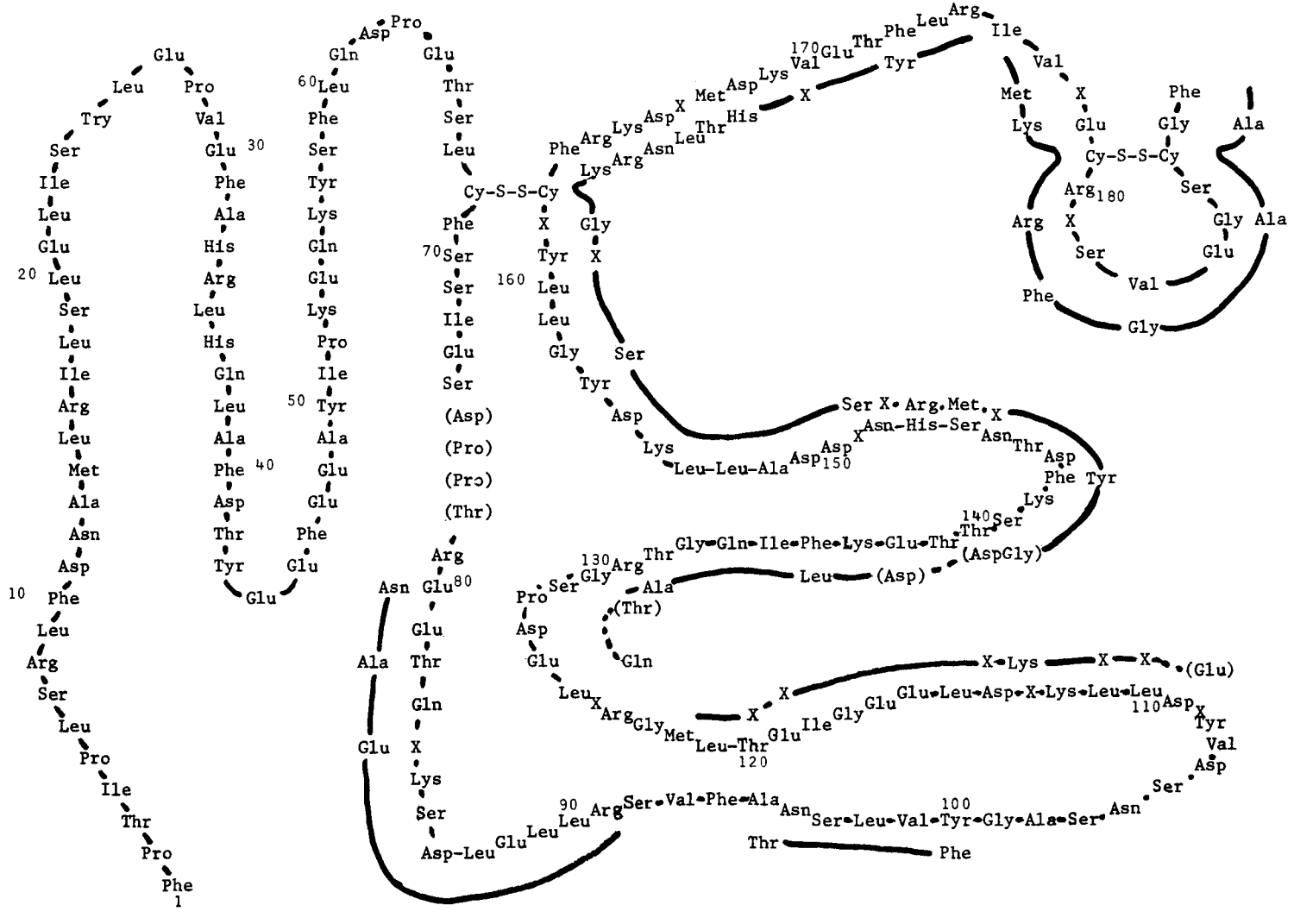


Figure 1 -- HGH[50] & BGH[52]

Substitution of a C-terminal prolinol for the Pro residue yielded a nonadecapeptide with MSH activity similar to that of the corresponding nonadecapeptide amide or native ACTH. ACTH activity was higher than that of the amide and lower than that of native ACTH, suggesting that the C-terminus structure has a separate effect from the basic core's net charge on ACTH function.[60] $Arg^{(8)}$ is essential for ACTH and MSH function of the 1-17 heptadecapeptide amide. Lys^{8}1-17 ACTH amide had much lower activity.[61] ACTH elevates intracellular cyclic 3',5'-adenosine monophosphate (AMP) which may mediate ACTH-induced adrenal steroidogenesis.[62] D-Phe^{7}1-10-ACTH was reported to facilitate extinction of a learned response when injected into rats, whereas the injection of the molecule containing L-$Phe^{(7)}$ appeared to inhibit extinction.[63] Certain synthetic amino acid sequences related to both ACTH and MSH raise blood ^{131}I levels in mice, suggesting increased thyroid activity.[64] The immunologic activity of ovine ACTH and α-22-39 ACTH were almost abolished by carboxypeptidase treatment whereas steroidogenic activity of ACTH was unchanged.[65] The synthetic full chain β-1-39 ACTH is antigenic, whereas the 1-24 fragment is virtually not;[66] a fact which may explain the clinical observation that β-1-24 ACTH does not provoke hypersensitivity.

Lipotropins.-Many substances with lipid-mobilizing activity have been isolated from anterior pituitary tissue of various species.[67,68] The term "lipotropin" (LPH) refers especially to the series of peptides isolated by Li and his associates during the course of purification of sheep ACTH. The first compound isolated[69] was designated L' and was reported to have MSH activity and lipolytic activity in rabbits but not in rats. L' contained 59 residues with Glu as the N-terminus. Ovine and bovine β-LPH were isolated[70,71] next and found to have essentially the same amino acid composition, mol. wt. and biological activity including low-order adrenocorticotropic activity. A side-fraction from the isolation of ovine β-LPH yielded γ-LPH which exhibited similar biological activity, but was of lower mol. wt.[72] The complete sequence of both peptides was elucidated.[70,73] β-LPH (Fig. 2) was shown to be a single chain polypeptide of 90 residues with Glu as the N-terminus and Gln as the C-terminus. γ-LPH was identical to the N-terminal 58 residues of β-LPH and the complete octadecapeptide sequence of ovine β-MSH was represented by residues 41-58. The low-order adrenal-stimulating activity was accounted for by residues 47-53 which are the same as residues 4-10 of ACTH. These interesting discoveries, along with previous knowledge, reveal the possibility of a mechanism for protein synthesis which involves either synthesis of a number of peptides with overlapping identical sequences or the production of larger molecules containing multiple active sequences which may be broken down selectively to provide an individual hormone.

Other Pituitary Hormones.-Continuing research on melanocyte-stimulating hormone (MSH) has been well-summarized in three 1967 reviews.[74-76] Some new structure-activity relationships have been reported[77] on synthetic C-terminal decapeptides related to β-MSH of several species. The total synthesis of monkey β-MSH was reported.[78] A radioimmunoassay was developed[79] for β-MSH and claimed to be capable of distinguishing β-MSH from α-MSH and ACTH. β-MSH was found to protect rats exposed to whole-body x-irradi-

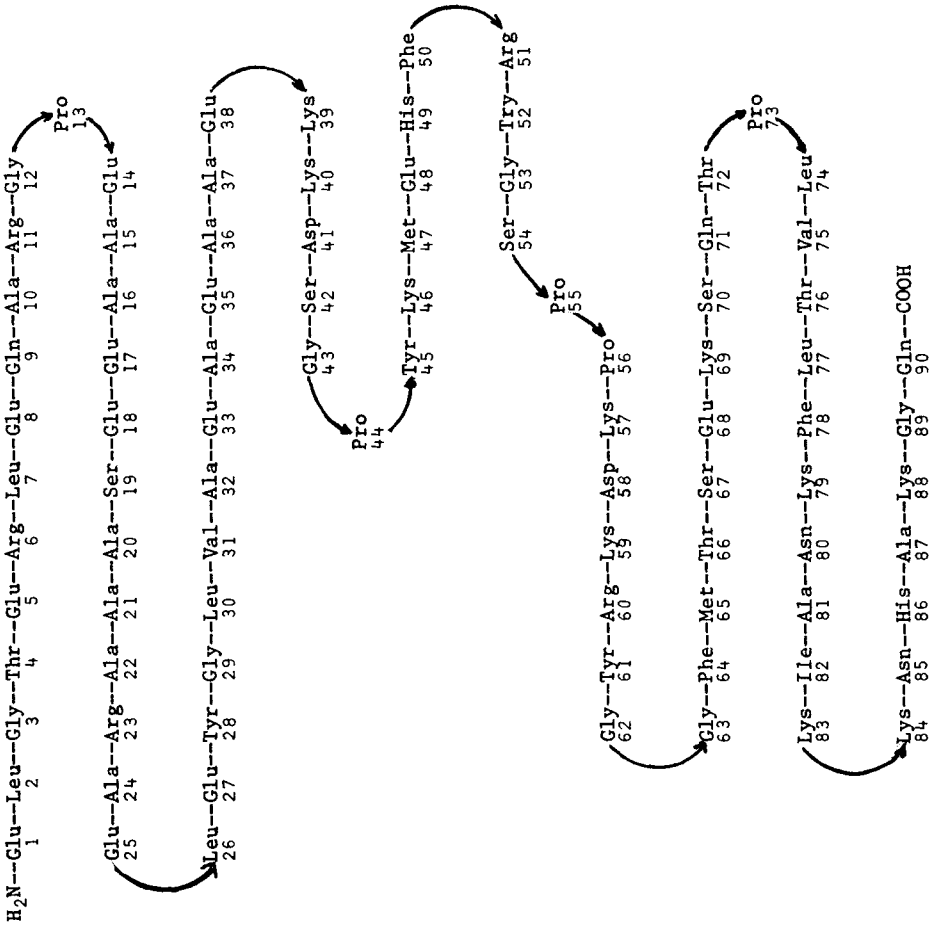
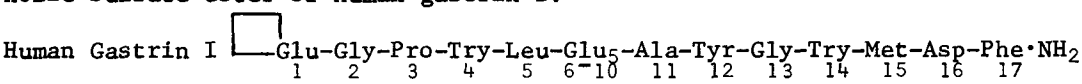


Figure 2 -- β-Lipotropin[73]

ation.[80] Important advances have been made in the purification and characterization of prolactin, luteinizing hormone, follicle-stimulating hormone and thyroid-stimulating hormone of various species, but as yet the complete amino acid sequence of none of these pituitary proteins has been reported. Oxytocin (OXT) and vasopressin (VP), the major neurohypophyseal hormones, which are presumed to be synthesized in the hypothalamus and released from the posterior pituitary, have continued to receive the attention of many investigators. In addition to a general compilation on active peptides,[81] a review[82] and a symposium[83] cover structure-activity relationships of broad classes of OXT and VP analogs. The original list of six naturally occurring hormones of this type (Arg-VP, Lys-VP, OXT, Arg-vasotocin, isotocin, mesotocin) has been expanded to include glumitocin ($Ile^3Ser^4Gln^8$ VP).[84] The question of independent biosynthesis[85] and release[86] of VP and OXT is under study along with the role of the carrier protein neurophysin.[87]

HORMONES OF THE STOMACH

Gastrin.-The gastrins[6] are a group of polypeptide hormones which are released in the gastro-intestinal tract of man and certain other species, and can be extracted from the antral region of the stomach. They have pronounced physiological actions on secretory cells of the stomach and pancreas, and on the musculature of stomach and intestine. Kenner et al. at the University of Liverpool and Morley et al. of Imperial Chemical Industries, Ltd., have continued their studies on hog and human gastrins. The sequence of human gastrin I has been determined and shown to differ from that of porcine gastrin I only in having Leu at position 5 instead of Met. As in the case of the porcine hormones, human gastrin II is the phenolic sulfate ester of human gastrin I.[88]



Total syntheses of these heptadecapeptide hormones have been reported[89,90] in detail. The physiological properties of a large series of synthetic peptides structurally related to the gastrins indicated that only the C-terminal tetrapeptide sequence was essential for most of the remarkable range of physiological effects of the natural hormones.[91] Of all the synthetic peptides studied, N-t-butyloxycarbonyl-β-Ala-Try-Met-Asp-Phe-amide, now known as pentagastrin (Pentavlon) was selected for extensive studies in animals and man. Because of the valuable properties found, this peptide was made commercially available by ICI for use as a diagnostic agent in the study of gastric secretion.[92] Studies in man showed that a single subcutaneous dose of pentagastrin could be used instead of histamine in assessing "maximal" acid response.[93,94] Wormsley[95] reported that at 5x the subcutaneous dose pentagastrin was equally effective when administered by nasal insufflation. Many studies[96-99] were reported on the comparison of the activities of the synthetic gastrin analogs vs. gastrins I and II and vs. histamine. In general, the actions are clearly distinguishable from those of histamine and the actions of the synthetic peptides are identical with those of the natural hormones. However, Neely[100] reported that pentagastrin failed to produce a motility response in the small intestine and

that the effect of atropine on the actions of pentagastrin and gastrin differed. Continuous I.V. infusions of porcine gastrin II was reported[101] to induce gastric and duodenal ulcers in conscious cats. Similarly, pork gastrin administered with gelatin produced duodenal ulcers in the guinea pig.[102] Antibodies to the gastrins have been prepared[103,104] and studies with them suggest that the C-terminal tetrapeptide which constitutes the physiologically active site is accessible on the surface of the intact hormone and determines the specificity of the antibody. 2-Phenyl-2-(2-pyridyl) thioacetamide ("antigastrin") has been reported[105] to be a specific inhibitor of both gastrin II and pentagastrin, but to have no effect on response to histamine or methacholine.

Secretin.-In spite of the fact that the term HORMONE was coined in connection with the discovery of secretin by Bayliss and Starling in 1902, very little definitive information was known about the chemistry of this substance until recently. Mutt et al.[106] reported the amino acid composition and partial sequence of porcine secretin in 1965. Soon after this they proposed[107] a tentative sequence for the 27 amino acid residues and in October, 1966, Bodanszky et al.[108] announced the total synthesis. Detailed reports[109,110] of the synthesis are now available and the synthetic heptacosapeptide amide possesses the characteristic biological activities of the natural porcine hormone. Some ambiguities regarding specific activity apparently still exist, but samples of the synthetic product were indistinguishable from natural secretin in paper chromatographic behavior, quantitative amino acid analysis, and enzymatic cleavage products.

Porcine Secretin	His	Ser	Asp	Gly	Thr	Phe	Thr	Ser	Glu	Leu	Ser	Arg	Leu	Arg	Asp	Ser	Ala	Arg	Leu	Gln	Arg	Leu	Leu	Gln	Gly	Leu	Val	-	NH_2
	1				5					10					15					20					25		27		

The classic action of secretin was to stimulate a copious flow of pancreatic juice with a high bicarbonate content and low enzyme concentration. With the availability of purified secretin evidence for a gastric inhibitory effect was reported.[111,112] Recently, Unger et al.[113] have reported that both secretin and pancreozymin have insulin-releasing activity. Boyns et al.[114] suggest that although secretin is insulinotropic it is so only in unphysiological doses.

HORMONES OF THE THYROID AND PARATHYROID

Calcitonin.-In 1962 Copp et al.[115] postulated the existence of a hypocalcemic hormone from the parathyroid and named it "calcitonin." Shortly thereafter Hirsch et al.[116] discovered a hypocalcemic, hypophosphatemic principle in thyroid tissue and named it "thyrocalcitonin." Subsequently, it was shown that the two activities are identical and that strictly speaking the hormone is produced by parafollicular cells which are embryologically analogous to the ultimobranchial bodies of lower species.[117,118] Calcitonin acts directly on bone by inhibiting resorption. Its action with regard to calcium is opposite to that of parathyroid hormone; with regard to phosphorus, the action is the same as that of parathyroid hormone and additive.[118] The hormone appears to be a peptide of low molecular weight.

Highly purified preparations of porcine calcitonin have been reported;[119-124] but there seems to be no general agreement regarding amino acid composition and no sequence has been reported. There is one claim that the hormone contains iodine.[125] Highly purified human calcitonin has been prepared and reported to induce a sustained reduction of serum Ca levels in hypercalcemic patients.[126] Although porcine calcitonin has been shown[127,128] to be hypocalcemic in man and calcitonin derived from several species is active in the rat, there is evidence to suggest at least some species specificity for the hormone.[129,130] This will undoubtedly continue to be a very active field of research and the therapeutic potential of calcitonin in osteoporosis, metabolic bone diseases and Ca homeostasis will be evaluated in the near future.

Parathyroid Hormone (PTH).-Arnaud, Tenenhouse and Rasmussen[131] have published a review on the Ca-sparing hormone PTH which represents a survey of the literature through June, 1966. Early studies on the isolation and characterization of PTH were done mainly with material of bovine origin.[132,133] Recently porcine PTH has been isolated[134] and found to be similar to the bovine hormone in biological activity and amino acid composition. The minimum mol. wt. indicated by ultracentrifuge measurements is 11,100 and by amino acid composition, 9,622 for the porcine hormone. These values are 11% and 15%, respectively, larger than the values determined for bovine PTH. Potts et al.;[135] on the basis of partial degradation studies, have proposed that the minimum portion of bovine PTH requisite for biological activity constitutes a C-terminal sequence of ca 20 amino acids with a slightly smaller segment of the same region being important for immunological reactivity. The following presumptive partial sequence for the C-terminal portion of the molecule was suggested: (Tyr,Gly,Arg)Try-Lys-His-Ile-Met-Glu-Ser-Phe-Ala-Val-Leu-Gln. Removal of the 4 C-terminal residues caused no loss of activity but alteration of Met, Try and Tyr at positions 77, 74, and 65, respectively, caused marked inactivation. Virus-transformed human parathyroid adenoma cells were reported to produce PTH for long periods as a monolayer culture.[136] A radioimmunoassay has been developed.[137]

HORMONES OF THE PINEAL

The pineal is now recognized as a vital component in the integration of light-mediated CNS activity with hormonal regulatory mechanisms. The prototype family of methoxyindoles represented primarily by melatonin (MT), is produced only in the pineal in mammals because it alone contains the enzyme hydroxyindole-O-methyl transferase (HIOMT) which transfers the methyl group from S-adenosylmethionine to the 5-hydroxy (5-H) group of a variety of indoles, including N-acetylserotonin (its best substrate). The enzyme HIOMT is present throughout the CNS of amphibians[138] and although it is lacking in human peripheral nerve, its pineal product MT is found there, suggesting that it may be a true hormone.[139] In lower vertebrates, the pineal is a photoreceptor, transmitting information about environmental lighting directly to the brain.[140] In rats (and presumably in other mammals) there is no evidence for a direct pineal response to light, and yet continuous light markedly inhibits HIOMT activity.[141] This was explained by the finding that

central nervous pathways[142] carrying photic impulses from the retina connect with and activate sympathetic nerve fibers originating in the superior cervical ganglion[143] and terminating on pineal parenchymal cells. In addition to MT, serotonin (5-HT), norepinephrine (NE) and histamine are found in high concentration in the pineal[144] with much of the 5-HT and all of the NE being localized within the sympathetic nerves.[145] A circadian 5-HT rhythm[146] is abolished by cervical sympathetic ganglionectomy.[147]

5-HT occupies an important position because of its dual localization in serotonergic nerves and pineal parenchyma[148] and because of light regulation of its metabolism, which leads to the formation of 5-H and 5-MeO-tryptophols and 5-H and 5-MeO indoleacetic acids. Cyclic derivatives of the carbolin (5-MeO-TOL) type may also be derived from these compounds. MT was characterized in 1959[149] as a principle in bovine pineal extracts which lightens the color of frog melanocytes. The pineal enzymes tryptophan hydroxylase[150] and aromatic amino acid decarboxylase[151] synthesize 5-HT from Try. Most of the 5-HT formed in the pineal from Try and 5H-Try is oxidized to 5-H-indoleacetic acid (5-HIAA)[152] by monamine oxidase (MAO)[153] and aldehyde dehydrogenase. A portion of the indoleamine is N-acetylated[154] and converted to MT by HIOMT. Pineals in organ culture transform 5-HT to 5-HIAA and to MT.[155] The release of MT is under direct cervical sympathetic control by way of nervous impulses whose frequency and pattern are modulated in a circadian fashion by fluctuating external light and retinal illumination.[156] Axelrod *et al*.[157] found that the weight of the pineal and the activity of the MT-forming enzyme HIOMT were greater in chickens exposed to continuous light or to diurnal light than in those exposed to continuous darkness. Because light stimulates pineal MT and avian gonadal activity, MT, which has an inhibitory influence on reproductive function in the white rat,[158] is probably induced in female birds by photoperiods which also accelerate gonadal development. Therefore, in rats, light cycles, acting by way of the retina through cervical sympathetics, maintain an inhibition of pineal antigonadotrophic (MT) activity.

MISCELLANEOUS BIOLOGICALLY ACTIVE SUBSTANCES

The kinins,[6] long known to be widely distributed in living tissue, are smooth muscle stimulating, vasodepressor, pain-producing peptides which act as local hormones that mediate natural defense reactions to injury. They are liberated from plasma protein by enzymes called kallikreins which require activation by the blood-clotting factor XII (Hageman factor). Major advances in the understanding of the role of these tissue hormones in physiological and pathological phenomena are summarized in recent symposia[159,160] and reviews.[161,162] Other naturally occurring peptides such as eledoisin and physalaemin[6] have actions similar to the kinins. Although modifications[163] of these structures have been made (mainly to improve the hypotensive activity) apparently no clinically useful compounds have been discovered. Caerulein,[164] isolated from the skin of certain amphibians, has potent and relatively long-lasting hypotensive activity and also striking action at the ng level on the gallbladder and on gastric and pancreatic sections.[165] This remarkable decapeptide possesses the activities of both the kinins and the gastrins and has the same C-terminal pentapeptide sequence as gastrin

II. The sequence of caerulein has been confirmed by synthesis.[166] Still other pharmacologically active peptides, e.g., melittin and apamin, have been isolated from insect venoms.[159,167]

Caerulein Glu(1)-Gln(2)-Asp(3)-Tyr(SO$_3$H)(4)-Thr(5)-Gly(6)-Try(7)-Met(8)-Asp(9)-Phe(10)-NH$_2$

Angiotensin II,[6] the potent vasoconstrictor and pressor octapeptide released from a plasma α_2-globulin by the kidney enzyme, renin, continues to receive considerable research attention. A comprehensive review[168] of the chemistry, structure-activity relationships and biology of the renin-angiotensin system has just become available. The structure of human angiotensin II was reported[169] to be identical to that of horse (Ile in position 5 rather than Val for bovine). Several radioimmunoassays have been reported.[170-172] The problems of achieving the pg sensitivity required for convenient determination of blood levels and specificity for angiotensin II do not appear to be completely solved.[173,174]

The antihypertensive function of the kidney and the role of the kidney as an endocrine organ have received increasing attention in recent years. At least three distinct types of compounds appear to be involved: 1) acidic lipids with short-term vasodepressor activity, 2) neutral lipids with long-term antihypertensive activity[175] and 3) phospholipids with renin-inhibitor and antihypertensive activity.[176] Of these only the first type has been characterized chemically. The acidic vasodepressor lipids of rabbit kidney were shown to be the prostaglandins, PGE_2 and PGA_2.[177] A distinguishing feature of the other two types is that they have no effect on the blood pressure of normotensive animals.

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Chapter 19. Reproduction

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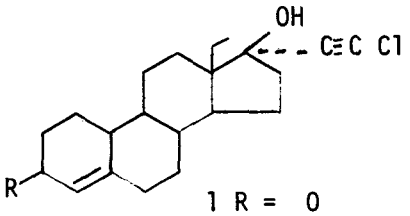
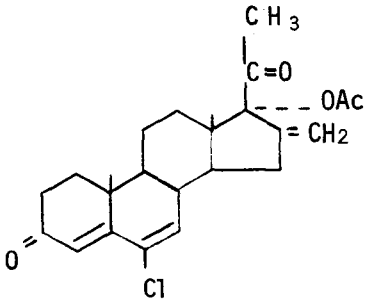
Introduction - The proliferation of oral contraceptive preparations containing combinations of progestins and estrogens continued unabated.[1] Intensive investigation of other drug-induced methods of reproduction control has not yielded yet a marketable product. Meanwhile, private and public funding for research on human reproduction problems continues to increase. But real progress in the control of human population growth may be achieved only when the minds of men are willing to acknowledge the horrendous realities of unchecked growth. The most singular contribution in the field of reproduction in 1967 may well have been the courageous and forthright concepts of population control put forth by Kingsley Davis.[2]

Steroids - The synthesis of ethynodiol diacetate and various analogues thereof now has been described in detail.[3] Two more potent progestins related to norgestrel, compounds 1 and 2, have been biologically evaluated.[4]



1 R = O
2 R = -OCOCH$_3$

The addition of a methyl group at C-18 of 19-norprogesterone enhanced its progestational and antiestrogenic activities whereas like treatment of progesterone decreased its progestational activity while increasing its antiestrogenic activity.[5] The biological activities of two progestins derived from chlormadinone acetate, compounds 3[6] and 4[7] have been described. The A-norsteroids 5 and 6 are progestational[8] and represent the first examples



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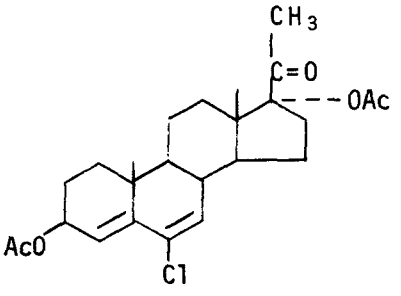
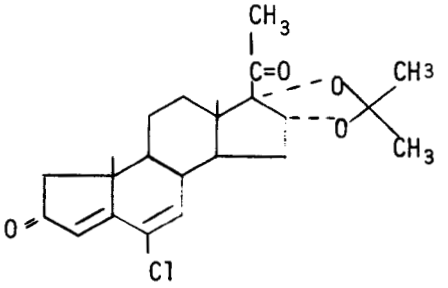
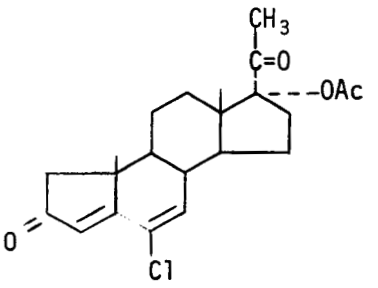


4

of A-norsteroids with this activity.

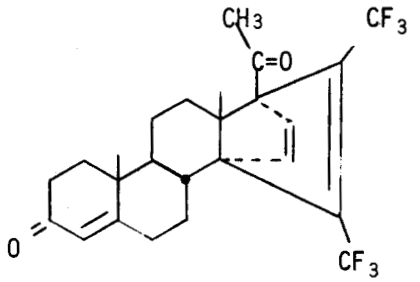


5

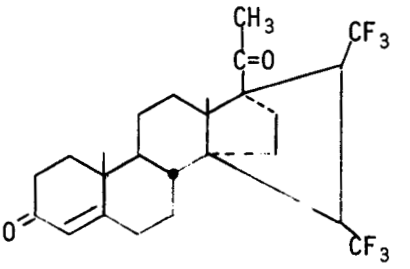


6

The progestational activity of 14α,17α-ethenopregn-4-ene-3,20-dione has been extended to the two interesting structures 7 and 8.[9]

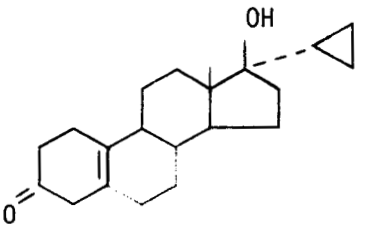


7

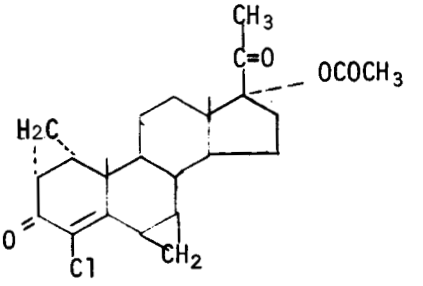


8

Substitution of a 17α-cyclopropyl group for the 17α-ethynyl group in a series of 19-norsteroids did not qualitatively change their biological activities.[10] Thus 9 is both estrogenic and progestational as is the 17α-ethynyl analogue, norethynodrel. Compound 10 is a strong progestin and ovulation blocker.[11]



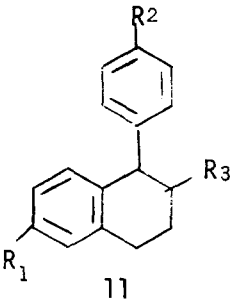
9



10

The use of a long-acting estrogen, 3-cyclopentyl ether of ethynyl estradiol, with a short-acting progestin such as chlormadinone acetate results in a long-acting contraceptive effect thus permitting a one-pill-a-month regime.[12] Another long-acting contraceptive effect was obtained by a single 150 mg. injection of medroxyprogesterone acetate every three months.[13]

Non-steroids - Interest in non-steroidal agents for reproduction control appears to be increasing. Several tetrahydronaphthalene derivatives of basic structure 11 have potent estrogenic and antifertility activity.[14]

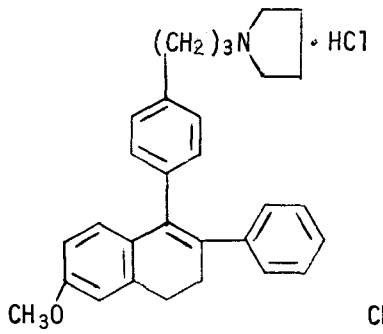


11

Quite unexpected is the uterotrophic potency of the hydrocarbon 11 (R_1 and R_2 = H, R_3 = C_6H_5). When R_1 = H, R_2 = $OCH_2CH_2N(C_2H_5)_2HBr$ and R_3= 4-ClC_6H_4 and the structure is trans the compound is a highly effective implantation inhibitor. Other active compounds in this series are those of structure 11 with the following substituents:

R_1	R_2	R_3
H	$OCH_2CH_2N(C_2H_5)_2$	H (cis isomer)
H	$OCH_2CH\underline{\overset{O}{\frown}}CH_2$	4-ClC_6H_4
H	$OCH_2CH_2N(C_2H_5)_2C_6H_8O_7$	2-FC_6H_4
H	$OCH_2CH_2N(C_2H_5)_2HCl$	4,5-$Cl_2C_6H_3$

The series of basic 3,4-dihydronaphthalenes and 1,2,3,4-tetrahydro-1-naphthols was extended[15] to afford two dihydronaphthalenes 12 and 13 and the naphthol 14 which are very potent antifertility agents in rat tests.



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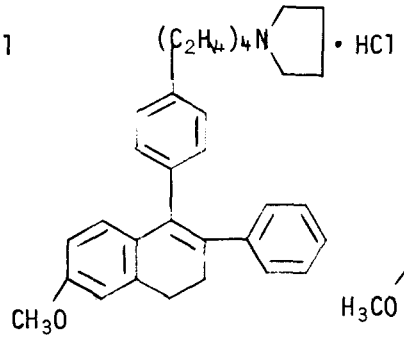

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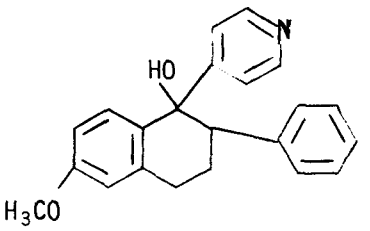
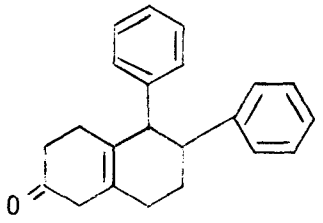


14

The reduced naphthaleneones 15, 16 and 17 are effective antifertility agents as indicated in rat studies.[16]



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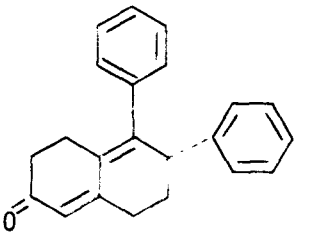

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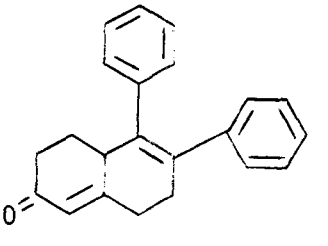
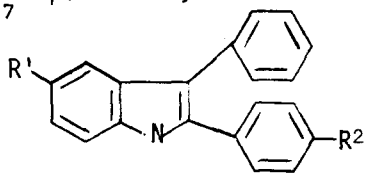
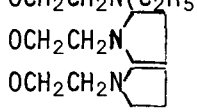


17

Compounds 18, 19 and 20 are effective implantation inhibitors in rats.[17]

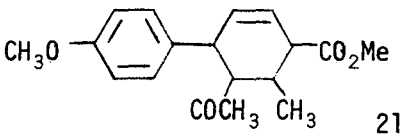


18 R' = H R^2 = $OCH_2CH_2N(C_2H_5)_2$

19 R' = H R^2 = OCH_2CH_2N 

20 R' = F R^2 = OCH_2CH_2N

The estrogenic activity and antifertility effect of fenestrel[18] and similar substituted cyclohexenes such as 21[19] and 22[20] were described.



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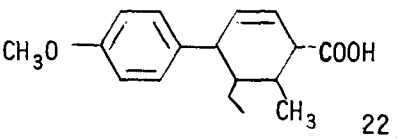
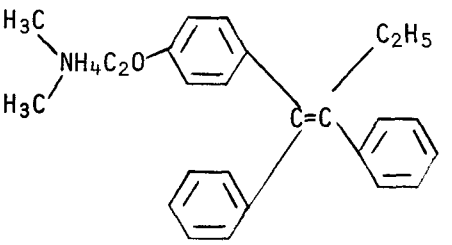


22

A compound similar to clomiphene was separated into its cis (I.C.I. 47,699) and trans (I.C.I. 46,474) structures (23 and 24 respectively) which were shown to have markedly different biological activities.[21]



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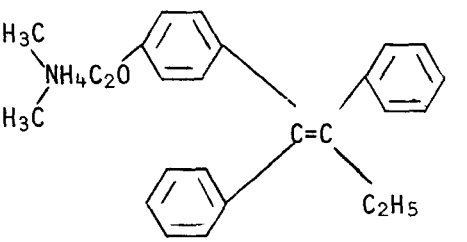
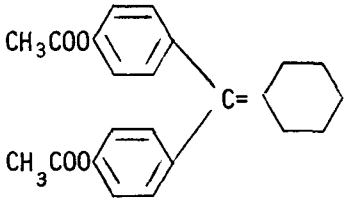


24

Thus 24 is orally active in inhibiting nidation in rats and is a weak and atypical estrogen with anti-estrogenic properties and little pituitary-inhibitory activity. The cis isomer 23 acts like a conventional estrogen. The synthesis of the much investigated clomiphene citrate was published.[22] F6066 (25) prevents conception when given to rats and mice

on day six or earlier after demonstration of spermatozoa in the animal.[23] No effect on pregnancy was observed when the compound was administered on day seven or thereafter. 2-Phenyl-3-diethylpyrrolidino ethoxy-6-methoxybenzofuran hydrochloride caused a 100% reduction of fertility in rats and mice when given on days 1-5 at 4 mg/kg orally.[24] The anti-fertility effect is presumed to be caused by premature expulsion of ova from the fallopian tubes. U-11634 (26) is not active as an antifertility agent by virtue of its antithyroid effect[25] and its mode of action still remains undetermined.



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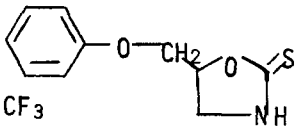

26

Natural Products - A single homogeneous protein component "blastokinin", isolated from the rabbit uterus and of estimated molecular weight 27,000, has been identified as an agent which induces blastulation of rabbit morula and stimulates blastocyst development.[26]

Decapacitation factor (DF) obtained from rabbit and bull seminal plasma has a molecular weight below 2000 and is estimated to be about 500 for the DF from the bull.[27] DF is said to be a naturally occurring antifertility substance since it does not possess toxicity for sperm but definitely prevents sperm from penetrating ova.

A review[28] of the possible effect of an unidentified factor or factors in wheat germ oil which may influence fertility again focuses attention on this long-studied material. Octacosanol, a 28 carbon straight chain saturated alcohol present in wheat germ oil may enchance fertility in some animal species. Further investigation is required.

A crude aqueous extract of powdered Combretodendron africanum bark is a smooth muscle stimulant and reportedly[29] has contraceptive as well as abortifacient properties. Another plant material with a fertility lowering effect on rats is byakangelicin, a furancoumarin from Ferula alliaceae.[30] A steroidal oily fraction extracted from the seed of Abius precatorius was orally active when tested for antifertility activity on rats and mice.[31] The material also produced 80% sterility in rats when administered as a single oral dose of 150 mg of oil daily for 1-5 days in the post coital period.

A report[32] on the use of human menopausal gonadotropin in conjunction with human chorionic gonadotropin or the use of clomiphene citrate in the treatment of anovulation in humans reveals that when patients are carefully selected for suitable therapy an overall pregnancy rate of approximately 56% may be achieved.

Reviews - A review of the clinical effects of antifertility agents appeared by Tyler.[33] The action of drugs on spermatogenesis has been reviewed by Fox and Fox.[34] Borth[35] reviewed current concepts concerning the endocrinology of the human menstrual cycle. The mode of action of contraceptive drugs has been comprehensively reviewed by Diczfalusy.[36] A recent bibliography on steroid conjugates[37] makes an excellent source for references to metabolic studies of oral contraceptives.

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Chapter 20. Steroid Hormones and Their Antagonists

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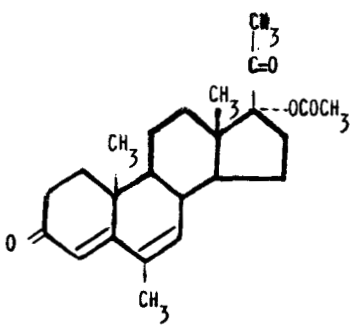
The proceedings of a conference on the activity of megestrol acetate (1) and of a symposium on quinestrol (14) have been published [1,2]. The abstracts of papers presented at the Sixth Acta Endocrinologica Congress in Helsinki are available [3], and so are the proceedings of the Fifth World Congress on Fertility and Sterility [4].

Progestational Agents - A biological classification of progestational agents comparing the various activities of this class of substances has been published [5]. The potent antiestrogenic effect of norgestrel (2) is discussed [5], and studies on its metabolic fate in humans have



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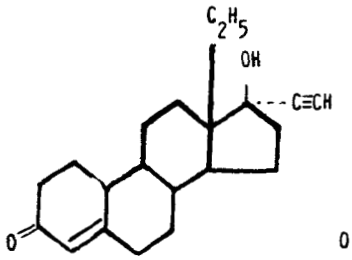

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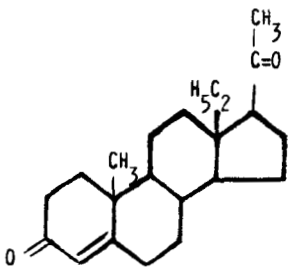
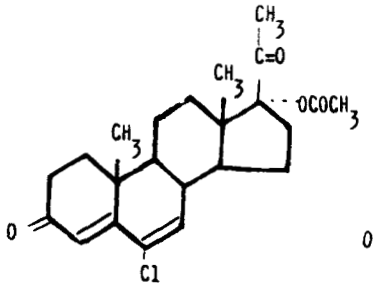


3

been reported [6]. The interactions of norgestrel with ethynyl estradiol (trade names of the combination: OVRAL and EUGYNON) have been described [7].

The synthesis and biological properties of racemic 18-homoprogesterone (3) have been reported [8].



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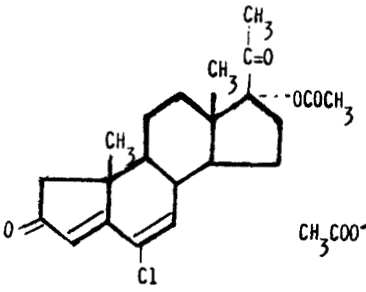

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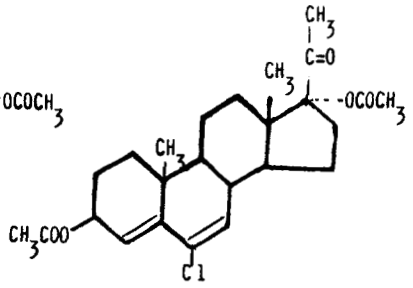
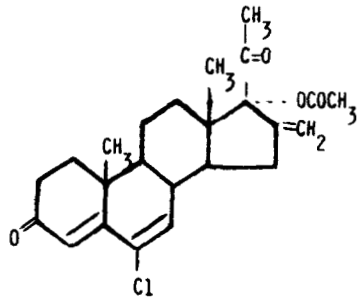


6

A dose relationship study with chlormadinone acetate (4) as a "minipill" had been published[9]; a clinical study[10] of this antifertility agent, and a novel approach in the contraceptive field, a reverse-sequential regimen aimed to improve the acceptability of 4 as a minipill has been described[11].

The synthesis of 5, the A-nor analogue of 4, has been recorded[12]. When compared in animals to chlormadinone acetate (4), the potent progestin AY-11,440 (6) displayed biological differences whose practical significance may be understood only after human trial[13].



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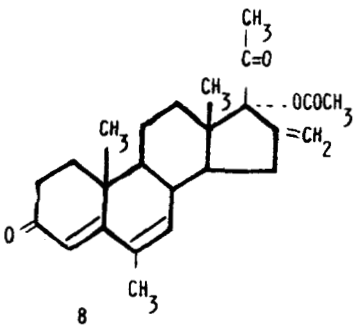

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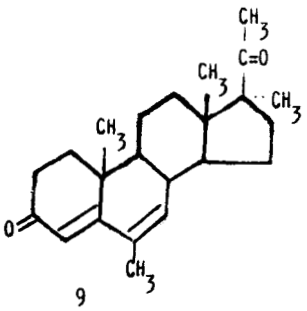
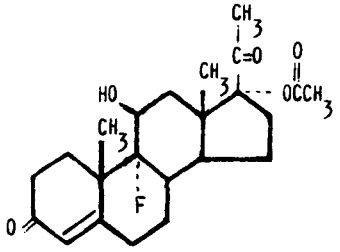


9

Maintenance of pregnancy data in animals for the 16-methylene analogue 7 have been reported[14] and so was the quantitative determination of melengestrol acetate (8) in bovine tissue[15].

The remarkable antitumor properties of medrogestone (9) on uterine myomas have been announced [16], and additional pharmacological evaluation was published [17].

The Nobel prize lecture by Huggins on endocrine-induced regression of cancers was printed [18].



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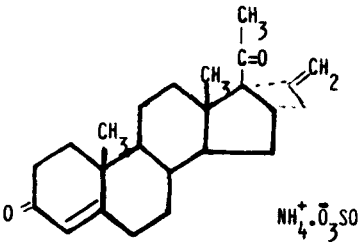

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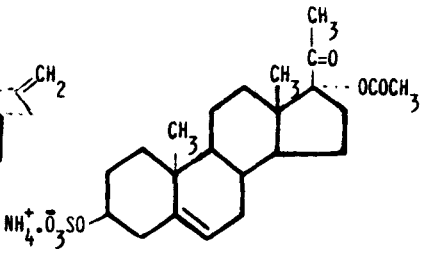
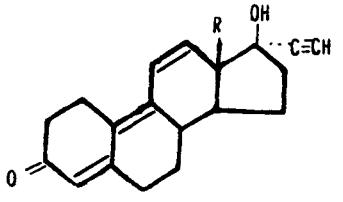


12

Experiments on the synchronisation of estrus in sheep with Cronolone (10) have been reported [19]. The synthesis and biological activity of the cyclobutane photoadduct 11 has been discussed [20].

The metabolic clearance rate in man and in rabbit of 17-acetoxypregnenolone sulfate (12), an orally active and water soluble progestin, has been studied [21].

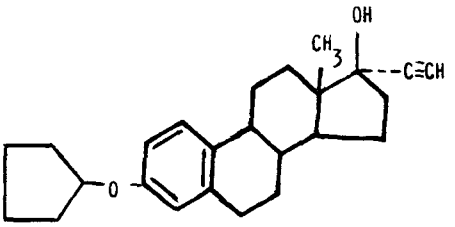


13

R = CH_3
CH_2CH_3
$CH_2CH_2CH_3$

The properties of homologous trienic steroids of structure 13 have been reviewed [22, 23].

Estrogens -



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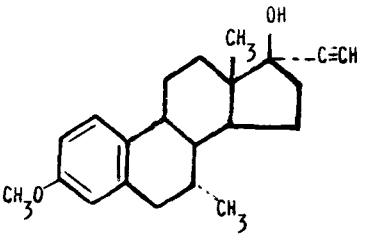
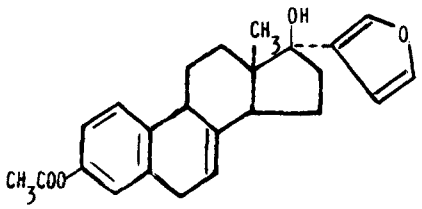


15

In addition to the various papers presented at a symposium on quinestrol (14)[2], a study on the distribution of radioactivity in body fat and organs of rats treated with this labelled estrogen has appeared[24].

In line with many other estrogens, the introduction of a 7α-methyl group into mestranol to give (15) enhances its estrogenic activity[25].



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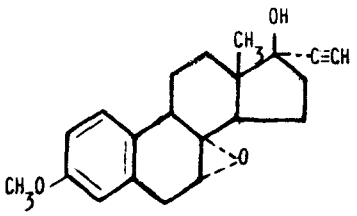
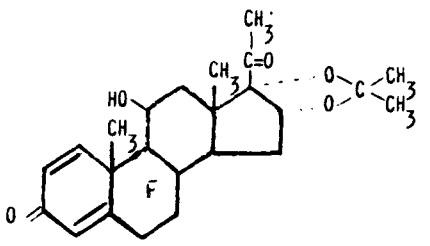


17

Two new series of equilin derivatives (16 and 17) of high estrogenic potency and interesting dissociation of certain estrogenic properties and uterotrophic activity have been reported[26, 27].

The exceptionally high potency of a new synthetic estrogen, B.D.H. 6197, reported to be 250-300 times more potent than ethynylestradiol, was briefly mentioned[28]. B.D.H. patents on substituted 17α-butadiynyl derivatives of estradiol have appeared.[29]

Corticoids -



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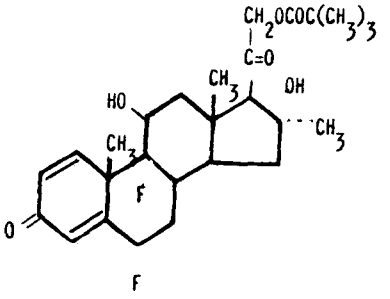
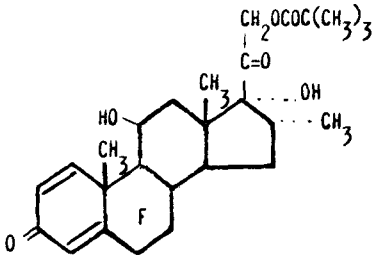


19

The biological activities of the 21-deoxy analogue 18 of triamcinolone acetonide have been published[30]. This steroid had interesting long-lasting properties upon intraarticular injection.

The results of a clinical investigation of a new formulation consisting of flumethasone pivalate (19) and coal-tar for the treatment of psoriasis have been published[31].



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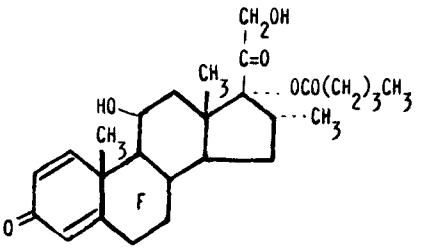
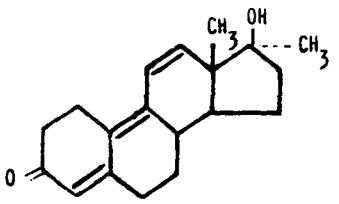


21

Dexamethasone pivalate (20) was successfully employed parenterally in patients with chronic rheumatoid arthritis[32]. The use of betamethasone 17-valerate (21) in the treatment of dermatoses was reported[33].

The synthesis and properties of various 17α-alkyl corticoid analogues appeared in press[34].

Androgens - Anabolics - Miscellaneous Steroids -



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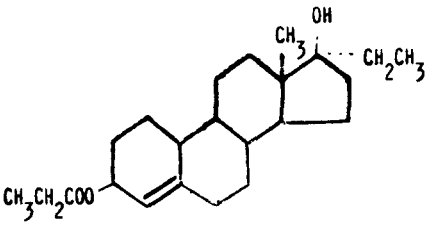
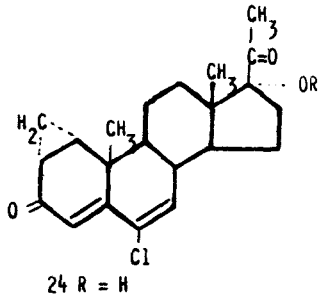


23

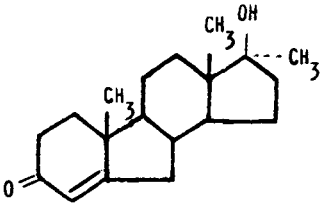
The clinical pharmacology of the anabolic trienolone 22 was investigated[35].

The effect of LSD-25 in man were reduced by the steroid SC-7294 (23)[36].

Antagonists - Several publications have appeared describing the interesting antiandrogenic properties of cyproterone (24) and its acetate (25).[37-41]



24 R = H
25 R = Ac



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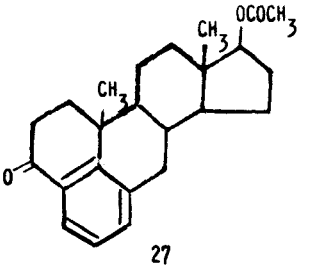

27

A report[42] on the antiandrogenic activity of the B-nortestosterone derivative (26), and on the anti-tumor compound (27)[43] are significant contributions in the field of steroidal antagonists.

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Chapter 21. Non-steroidal Anti-inflammatory Agents

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Introduction - With the introduction of several new nonsteroidal drugs over the past three years, the clinical application of anti-inflammatory agents has become widely accepted. Indications are that the trend will continue to increase and a superior agent, both from the potency and safety point of view, is still welcome. Several compounds mentioned in previous reports have gradually advanced to the clinical or marketing stage. A heightened interest in anti-inflammatory research is evidenced by the occurrence of three international symposia on the medicinal chemistry of these agents within a year or so.[1] Among the voluminous publications, another comprehensive review summarizing some seven hundred literature articles up to 1964 has appeared as a convenient reference.[2] The proceedings of a conference on Chemical Biology of Inflammation have just been published[3] to highlight some current investigations related to the initial phases of the inflammatory response.

Etiology and Immunological Aspects - The possible role of microorganisms and viruses in the etiology of arthritis continues to receive some attention.[4,5] No simple correlation between any infection and rheumatoid arthritis has been observed, but the presence of Bedsonia organism in urethritis and Reiter's disease appears more than a coincidence.[6] It seems not unlikely that many cases of autoimmune diseases may be the consequence of a genetically predisposed immunological abnormality precipitated by a diversity of infectious agents,[7] including hypothetical "slow viruses."[8] This kind of two-stage process has been postulated for various chronic degenerative disorders and presumably is facilitated by states of immunologic deficiency such as aggammaglobulinemia.[8]

Extensive literature on autoimmune diseases, e.g. clinical aspects,[9] animal models[10] and chemical suppression,[11] is now available. However, the immunological characteristics of many inflammatory disorders is still poorly understood. Traditional anti-allergy agents such as antihistamines and antiserotonins are inadequate for chronic inflammatory diseases like rheumatoid arthritis. Recent experiments with antimetabolites and cytotoxic agents capable of inhibiting cellular proliferation or antibody synthesis, e.g. cytoxan,[12] immuran,[13] methotrexate[14] and chlorambucil[15] have demonstrated certain degrees of clinical efficacy but often within a narrow range of therapeutic safety. Should a less toxic agent become available some reorientation of both patients and clinician regarding the benefit and long term risk involved in the use of immunosuppressants may still be needed.

The juxtaposition of a complex network of immune responses with an equally dynamic inflammatory process is still too speculative to offer any reliable approach to antirheumatic drugs. However, with the current advances in immunology and with the discovery of novel agents to unravel immune mechanisms at the cellular and molecular levels, a promising future in this new field of anti-rheumatic research is expected.

Biochemistry of Inflammation - The relationship between anti-inflammatory activity and the uncoupling of oxidative phosphorylation[16] is still being clarified. The degranulation of mast cells which releases histamine, serotonin, proteases and heparin, requires an energy supply either by oxidative or glycolytic pathway. A fair correlation between the anti-edema potency, uncoupling activity and inhibition of degranulation was demonstrated with a number of nonsteroidal agents.[17] On the other hand, the increase of ATP level in inflamed tissue was not affected by sodium salicylate at its anti-edema dose. If significant changes were indeed produced by drug treatment either the cells affected represent a minor constituent of the tissue or an unknown compensation mechanism is implicated.[18]

Previous considerations about the inhibition of histidine decarboxylase,[19] 5-hydroxytryptophan decarboxylase[20] and chymotrypsin[21] by anti-inflammatory agents have been extended. Several serum proteins such as necrotizing factor and complement are stabilized by low concentrations of these agents toward heat denaturation.[22] These observations further strengthen the view that since the observed biochemical changes in inflammation are only quantitative in nature clinically effective anti-inflammatory drugs are likely to be multivalent in their actions. Some of these in vitro systems probably are useful as primary screens capable of testing large numbers of small samples. Of course, any in vitro assay is subject to limitations which will yield many false positive results not correlated to in vivo activities. The uncertainty of a proposed in vitro anti-inflammatory assay, the binding of 2,4,6-trinitrobenzaldehyde to plasma albumin, was critically demonstrated recently.[23]

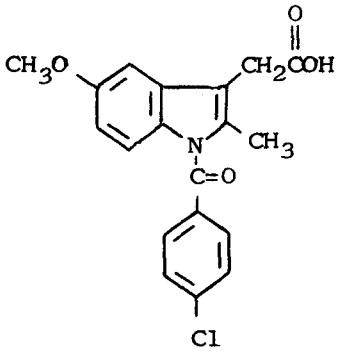
At the cellular level, anti-inflammatory agents stabilize erythrocyte membrane in hypotonic solutions[24] and against thermal shock[25] partly by causing membrane expansion up to 19%.[24] For aryl acids the protection is favored at lower pH, a condition which is ideally met in inflamed tissues and distinguishes cationic membrane stabilizers.

The well known cytotoxic properties of many anti-inflammatory drugs have assumed a positive role in the current search for immunosuppressive agents. Interest in lymphocytes was accentuated by recent investigations of antilymphocytic serum (ALS), which strongly discriminates against sensitized lymphocytes

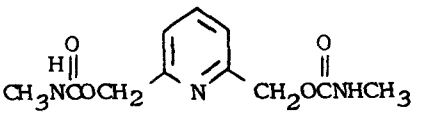
involved in cell-mediated delayed hypersensitivity.[26,27] Common anti-inflammatory agents at 10-50 γ/ml inhibit protein and nucleic acid synthesis in lymphocytes and epithelial cells to a varying degree.[28] Selective antilymphocytic property was demonstrated by indoxol although in a protein-free medium only.[29]

The close association of fibrin deposition and inflammatory response is further supported by several recent experiments. The carrageenin-edema in the rat paw is inhibited by agents such as pival and heparin which affect the formation and resolution of fibrin.[30] The moderate in vitro fibrinolytic activity of anti-inflammatory drugs has been confirmed and attributed to their binding to the fibrin molecule and possibly the release of a fibrinolytic activator.[31] Salicylate and phenylbutazone also inhibit platelet aggregation and adhesion induced by fibrin and collagen fragments.[32]

In acute gouty arthritis the activation of Hageman factor is suggested as the initiating event followed by release of mediators, permeability changes and migration of leukocytes.[33] The significance of numerous mediators varies according to the nature of inflammatory response involved, whether it is an acute, immediate-immune or delayed hypersensitivity type. The lymph node permeability factor (LNPF) appears to have a major role in delayed hypersensitivity. It is affected by relatively high doses of indomethacin, (I) salicylate and pyridinol carbamate.(II)



(I)



(II)

A permeability factor similar to LNPF has been found in thymus[35] and a variety of chronic inflammatory joint lesions including rheumatoids.[36] Newly formed capillary vessels in granulomas are particularly sensitive to stimulation or damage by biogenic amines, kinins, etc.[37] A combination of precapillary dilation and post capillary constriction leads to edema and leakage of plasma protein. These effects are inhibited by anti-inflammatory agents at their therapeutic concentrations in vivo.[38]

In Vivo Assays - The adjuvant arthritis assay has gained a wide acceptance, and the serum alpha-2-glycoprotein was suggested as a parameter to measure the inflammatory response in this protocol.[39,40] The immunological component in the adjuvant arthritis assay is not affected by Zymosan and methyl palmitate which enhances or depresses the reticuloendothelial function, respectively.[41] However, potent interferon-inducers (and RES affectors) like statolon[42] and pyran copolymer[43] are very active. It is doubtful that these "antiarthritic" activities are in any way related to their antiviral properties. As expected, antilymphocytic serum (ALS) inhibits the development of adjuvant arthritis.[44] The activity of estrone remains to be clarified.[45] The diversity of inhibitors in this assay would suggest caution in relating in vivo activity to clinical significance.

In addition, several versions of arthritis in animals are now available. In a modified adjuvant arthritis protocol animals are dosed for the first three days only. Immunosuppressants like cytoxan and immuran are capable of inhibiting some early events and suppress the development of polyarthritis two weeks later.[46] A periarthritis model is produced by the activation of a latent PPLO infection by 6-sulfanilamidoindazole.[47,48] The pathological syndromes are somewhat different from those expressed in the regular adjuvant arthritis. There is also a connotation regarding the possible etiology of human arthritis. So far no major discrepancy in drug response has been shown by these two versions.

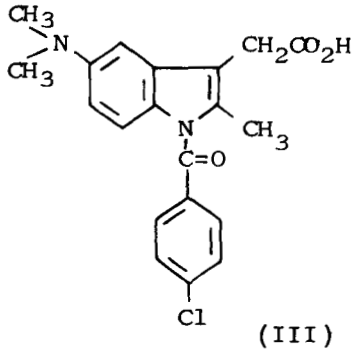
A comparison of adjuvant arthritis and allergic encephalomyelitis suggested that the latter may be a superior model to [49,50] evaluate the suppression of cell mediated delayed-hypersensitivity.

Polyene antibiotics such as filipin induce acute and chronic arthritis after repeated intra-articular injections in rabbit knee joints.[51] The histological lesions, presumably produced by lysosome disruption, resemble those in degenerative arthritis. Lesions produced by streptolysin S are considered to be rheumatoid arthritis-like.

An in ova assay capable of detecting the granuloma-inhibitory activity of various steroids and non-steroids, including chloroquin, has been developed.[52] However, the selectivity and other characteristics of this new protocol remain to be clarified.

Indomethacin and Analogs - While the clinical application of indomethacin in the treatment of spondylitis, gout, osteoarthrosis and rheumatoid arthritis remains widely accepted, its efficacy against rheumatoid arthritis has become a matter of some discussion in the recent literature.[53,54,55,56] For medicinal chemists the inadequacy of existing animal models to simulate rheumatoid arthritis should also be realized.

Additional mechanisms of action of indomethacin suggested are the inhibition of leukocyte migration[57] and antagonism of capillary constriction.[38] Like other anti-inflammatory agents it possesses moderate antilymphocytic properties in vitro, but no adverse effect on the host-resistance of experimental animals.[58,59]



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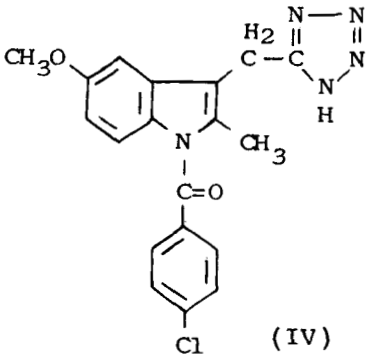
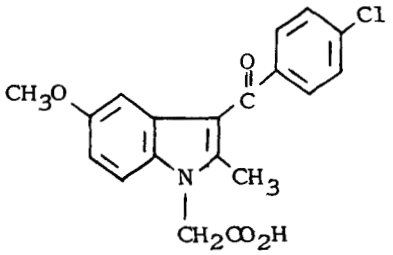


(IV)

A recently disclosed analog is the 5-dimethylamino derivative (III)[60] with a potency nearly 1/2 x indomethacin but with different metabolic and distribution properties.[61] The tetrazole analog of indomethacin (IV)[62] is about 1/20 to 1/10 as potent in animals and being investigated in the clinic. The rigid position isomer (V) was found previously to be much less active in animals at Merck Research Laboratories and this was confirmed independently in France.[63]



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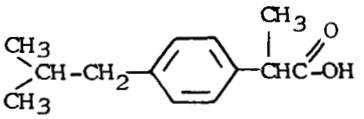
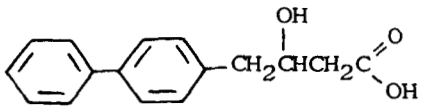
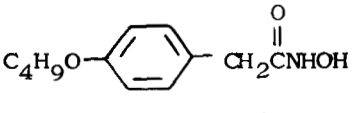


(VI)

Aryl Acetic Acids - The α-methyl homolog of ibufenac (Dytransin), ibuprofen (VI),[64] is 4 to 8 times more active in anti-inflammatory, analgesic and antipyretic assays,[65] but the increased potency was not confirmed in a brief clinical study.[66] Unlike other aryl acetic acids,[67] both d and l enantiomorphs are active in animals, although only dextro-rotatory metabolites with oxidized isobutyl side-chains were isolated as principle urinary metabolites.[65]



(VII)

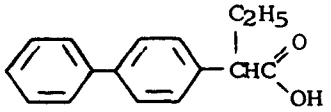


(VIII)

The potent anti-inflammatory activity of MJ (BDH)-1983 (VII) was discussed in detail,[68] but no clinical data has yet been disclosed.

Additional pharmacological results and the structure-activity relationship of p-n-butoxyphenyl acethydroxamic acid (VIII) (CP 1044 J 3, Droxaryl) have been published.[69] In man it is metabolized to the corresponding amide (CP 1044 J 4), the carboxylic acid and its glucuronide.[70]

With namoxyrate, the acid moiety (IX) is strongly bound by plasma proteins. It deposits largely in fat and muscle and to a smaller extent in the brain.[71] Contrary to expectations, the drug is not converted to a hydroxyl or glucuronide metabolite but excreted unchanged. The observed differences in distribution and metabolism of several aryl acetic acids indicate that comparative metabolic studies may be a practical approach to evaluate the long-term safety of many active aryl aliphatic acids.



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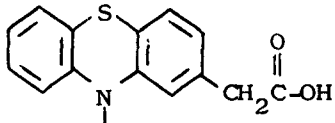
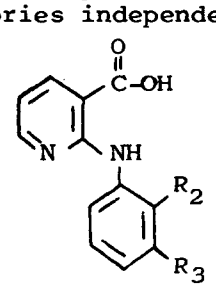


(X)

Another analog, 2-(10-methylphenothiazinyl) acetic acid (X) (16,091 R.P.) has shown a potency 3 times phenylbutazone in the carrageenin edema and U.V. erythema assay but less irritating in animals.[72]

Fenamates - N-Aryl anthranilic acid has become a major lead with broad appeal to many investigators. Several members of the fenamate family are actively being developed at the clinical stage. Mefanamic acid (Ponstel) was cleared by FDA for short-term use in the relief of pain,[73] and flufenamic acid (Arlef) is available abroad. The pyridine analogs were investigated by at least four laboratories independently.[74-77] Niflumic acid (XI) is comparable



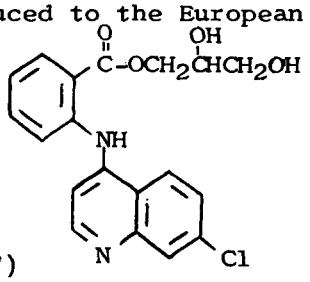
(XI) R_2=H, R_3=CF_3

(XII) R_2=CH_3, R_3=Cl

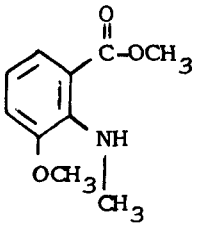
(XIII) R_2=CH_3, R_3=NO_2

to flufenamic acid in animals and is used in Europe at 1-1.5 g. per day. The 2-(2'-methyl-3'-chloro) analog (XII)[77] was reported to be similar in potency but less ulcerogenic in animals. The

2'-methyl-3'-nitro analog (XIII) was also claimed in a patent.[78] The phenothiazine analog, SKF 22908, reported last year is undergoing clinical study.[79] The quinoline analog (Glafenin, XIV) has been introduced to the European market as 200 mg. tablets.



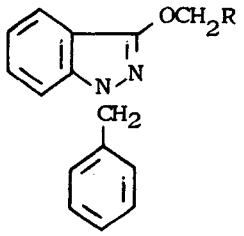
(XIV)



(XV)

Moderate antipyretic, analgesic and anti-edema activities were shown by the alkaloid damascenin, 3-methoxy-N-methylanthranilate, (XV)[80] again structurally related to a metabolite in the tryptophan pyrrolase pathway as are several antirheumatic drugs.

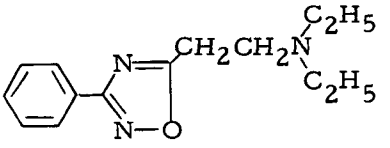
Analgesic Anti-inflammatory Agents - Inasmuch as a CNS component is involved in the development of inflammatory response, it is not surprising that morphine, chloropromazine, anti-depressants and related structures are highly active in the edema, granuloma and other anti-inflammatory assays.[81,82,83] A recent example was the alkaloid cryogenine[84] equipotent to phenylbutazone. These compounds usually produce less gastrointestinal irritation, but their application is more effective in acute inflammatory disorders and limited by other CNS effects associated with them.



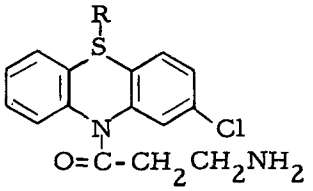
(XVI) R=$CH_2CH_2N(CH_3)_2$

(XVII) R=$C(=O)OH$

Benzydamine (Tantum) (XVI) was developed as an analgesic agent and its application in the clinic is being expanded.[85,86] The corresponding acetic acid (XVII) is only weakly anti-inflammatory,[87] again confirming that the aryl moieties optimal for carrying the anti-inflammatory acetic acid side-chain and the analgesic alkylamine side-chain are not usually identical. Oxolamine (XVIII) (Perebron) has been tried for treating inflammatory diseases of the urinary tract.[88] Its potential teratogenic effect was investigated.[89] Some related structures have also been examined.[90] Two 2-chloro-10-(β-alanyl)phenothiazines (XIX, XX) have shown anti-inflammatory and diuretic activity.[91] The sulfone derivative (XX) is 5 x hydrocortisone in the carrageenin edema assay.



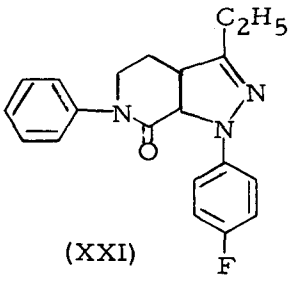
(XVIII)



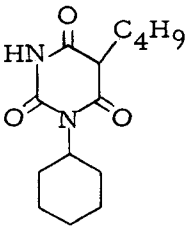
(XIX) R=O (XX) $R=O_2$

Many anti-inflammatory aryl acids possess peripheral antinociceptive effects; a ranking list of their relative analgesia in man and in animals has been compiled.[92] Perhaps to a lesser degree, their anti-inflammatory activity is also dependent on the CNS system in several assays.

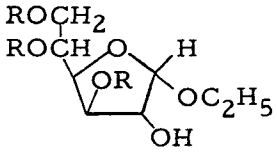
Miscellaneous - As a consequence of the wide-spread search for anti-inflammatory agents several novel structures with a variety of activity-profiles have been discovered. A pyrazolo(3. 4-c) pyridine derivative (Su 15335) (XXI), possessing the partial structure of a family of very potent steroids, has been reported to be one of the most active nonsteroidal agents.[93] 5-n-Butyl-1-cyclohexyl-2, 4, 6-trioxoperhydropyrimidine (XXII) (TBA 300) is a moderately active compound comparable to phenylbutazone.[94]



(XXI)



(XXII)



(XXIII)

R = -CH_2- 

The narrow anti-edema activity of trihydroxyethyl rutoside (z 6000) is inhibited by adrenalectomy.[95] Ethyl 3, 5, 6-tri-o-benzyl-D-glucofuranoside (XXIII) (CIBA 21401-Ba, Glyvenol) inhibits broadly the inflammatory mediators, the anaphylactic and acute inflammatory responses, the antigen-antibody reaction and the migration of granulocytes.[96, 97] The clinical application of this novel structure has not been published.

The efficacy of penicillamine and cysteine in the treatment of rheumatoid arthritis was considered modest only; further enhancement in potency and improvement of side-effects are needed. Sulfhydryl compounds possess an inhibitory effect on collagen synthesis[98] but their immunochemical properties remain to be elucidated.

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Section V - Topics in Biology
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Chapter 22. Drug Metabolism

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Introduction - The study of drug metabolism involves, in addition to the determination of the metabolic fate of the drug, studies of drug absorption and distribution, protein binding, transport across membranes, and drug excretion. As a result of such studies it is now apparent that most lipid soluble drugs are converted to other substances prior to their excretion mainly into the urine and the bile. Therefore, the therapeutic activity of a drug, its duration of action, and possible toxic manifestations will be influenced by the type and degree of its conversion in the organism.

The realization that some drugs owe their therapeutic effect to their metabolites has stimulated an interest in the study of the metabolic fate of new and older drugs in animals and man. Such studies have led to the development of several important drugs including the following "active metabolites"; the antibacterial agent sulfanilamide, a metabolite of prontosil rubrum; the analgesic p-hydroxyacetanilid, a metabolite of acetanilid; and the antidepressant desipramine, a metabolite of imipramine. Phenylbutazone is converted to two "active metabolites," oxyphenbutazone, the antirheumatic agent, and an uricosuric agent. It is expected that drug metabolism studies wil greatly increase in the years to come, partially due to the increasing emphasis by the Food and Drug Administration [1,2] on the usefulness of metabolic data in toxicity studies and preclinical investigations.

It will not be possible, in the space allotted, to review all the papers related to drug metabolism published during the last year, nor would such an extensive and detailed review be of much value to the medicinal chemists for whom this review is intended. The purpose of this communication is to present the basic concepts of drug metabolism and to illustrate these concepts by reviewing selected papers on drug metabolism published in 1967 in a fashion used previously [3,4].

It is hoped that a better understanding of metabolic pathways of drugs will stimulate the interest of the medicinal chemist to structurally modify the anticipated sites, or regions, of the metabolic attacks to permit synthesis of better and more

useful drugs. A number of excellent papers and reviews pertaining to drug metabolism appeared in the literature over the last few years to which the reader is referred [3-16].

General Aspects - The metabolism of a drug is dependent in a large degree on its physicochemical characteristics. Drugs with extremely low lipid solubility are generally not metabolized and are largely excreted in urine. Their excretion is dependent on the glomerular filtration rate, and they are not reabsorbed in the renal tubules. On the other hand, lipid soluble drugs diffuse from the glomerular filtrate back into the plasma until the concentrations of the unionized drug in plasma and urine are virtually identical [15]. Thus, the elimination of lipid soluble drugs by the kidney is extremely slow. Therefore, the major function of the drug metabolizing enzymes is to generate more readily excreted polar derivatives.

There exists in all species a general pattern for drug metabolism which can be described according to the following series of reactions [13]:

$$\text{DRUG} \xrightarrow[\text{reactions}]{\text{asynthetic}} \underset{\text{hydrolysis products}}{\overset{\text{oxidation, reduction and/or}}{}} \xrightarrow[\text{reactions}]{\text{synthetic}} \underset{\text{products}}{\text{conjugation}}$$

Most drugs are oxidized or reduced by enzymes located mainly in the endoplasmic reticulum of the liver cells. The rough-surfaced form of the endoplasmic reticulum contains ribosomes while the smooth-surfaced form is devoid of ribosomes, but contains the relatively nonspecific drug metabolizing enzymes. Small amounts of drug metabolizing enzymes are also located in the endoplasmic reticulum of the kidney, lung, and the gastrointestinal tract [10]. It is of interest that drug metabolizing enzymes are found in the liver of mammals, birds, and reptiles but are largely absent in fish and amphibia which dispose of foreign lipid-soluble compounds by diffusion through the gills or skin [5].

Oxidations are probably the most common reactions in drug metabolism. They are catalyzed by enzymes classified as mixed oxygenases because they require both a reducing agent (TPNH) and atmospheric oxygen. These enzymic reactions include aromatic hydroxylation, side-chain oxidation, N-dealkylation, O-dealkylation, sulfoxide formation, and deamination.

The present view of the chain of events leading to the oxidation of a drug by the microsomal enzymes has been summarized by Brodie [12] as follows:

$$\text{Drug} + \underset{(ox)}{\text{P-450}} \rightleftharpoons \underset{(ox)}{\text{Drug-P-450}} \xrightarrow[\text{P-450 reductase}]{\text{TPNH}} \underset{(red)}{\text{Drug-P-450}}$$

$$\xrightarrow{O_2} \underset{(red)}{\text{Drug-P-450}} \cdot O_2 \longrightarrow \text{Oxidized drug} + \underset{(ox)}{\text{P-450}}$$

A principle participant in the pathway is a newly described "component"—cytochrome P-450—which forms an "active oxygen" complex needed for the transfer of a hydroxyl group to the drug [10]. This "component," present in the microsomes of liver, kidney, and intestinal mucosa and in the adrenal microsomes and mitochondria has been characterized as a pigment which is unusual in that its spectrum in oxidized and reduced form is virtually the same, and when oxidized it does not form a complex with cyanide [10]. Its reduced form, however, readily combines with CO forming a complex with an absorption max. at 450 mμ (hence the name P-450).

Many types of drugs are metabolized by the liver microsomes to more polar derivatives; however such metabolism is not limited to drugs alone. Similarities have been shown between drug metabolizing enzymes and steroid hydroxylases in liver microsomes suggesting that steroid hormones can serve as substrates for the drug metabolizing enzymes [7].

A wide variability in the metabolic pathways exists between species, strains, sexes (primarily in the rat), and even between individuals. Most of these differences are quantitative in nature but some are qualitative. The reason for such variability is not as yet clear. It is possible that there may be a difference in the number and quantity of microsomal enzymes, or a difference in substrate specificity and other properties [10]. It is also possible that various types of enzymes are localized in different types of cells, thus the nature of metabolic transformation will be related to the amount and cell type present in the liver of a particular species or strain of animals [15].

Species differences in drug metabolism make a reliable extrapolation of drug behavior from animal to man very difficult and show the importance of carrying out metabolic studies in man. However, it has been shown for a number of drugs, that a valid projection of activity as well as toxicity from animal to man is possible when drug responses are related to plasma levels rather than dose [9,11,12].

Microsomal Enzyme Induction - The activity of liver microsomal enzymes can be altered by nutritional factors, hormones, and many foreign compounds. Drugs, insecticides, and carcinogens are known to stimulate drug metabolizing enzymes and frequently to increase the concentration of enzyme protein (induction) [16].

Enzyme induction is extremely important in evaluating pharmacological, toxicological, and therapeutic activity of a drug since it affects the duration and intensity of drug action.

There are at least two types of enzyme inducers [16,17]. The first, exemplified by phenobarbital, stimulates a large variety of metabolic reactions and causes proliferation of the smooth-surfaced endoplasmic reticulum [8]. It also exerts a marked anabolic effect on the liver resulting in an increased concentration of microsomal proteins [16]. The second, exemplified by the polycyclic aromatic hydrocarbon 3-methylcholanthrene, stimulates a limited group of enzymic reactions, has little effect on the proliferation of the smooth-surfaced endoplasmic reticulum and on the concentration of liver microsomal proteins, but stimulates liver growth and the synthesis of total liver proteins [16]. The mode of action of enzyme inducers is unknown , but synthesis of nucleic acids or proteins may be involved since the induction by phenobarbital and 3-methylcholanthrene is blocked by ethionine, puromycin or actinomycin-D. It is of interest that humans vary in responsiveness to microsomal enzyme stimulators and occasionally animals are found refractory. This suggests that a genetic factor may play a role in microsomal enzyme stimulation [16].

In addition to inducing the metabolism of other drugs, many substances have the ability upon chronic administration to stimulate their own metabolism. While some drugs like phenylbutazone and tolbutamide are rapid self-stimulators, others like probenecid may require 3 weeks to enhance their own metabolism [18]. The self-induction could account for changes in drug toxicity observed on prolonged treatment [9,18]. Welch et al [18] have recently suggested the use of the plasma half-life of antipyrine (which is completely metabolized and distributed evenly in body water) as an indicator of enhanced drug metabolism in animals.

Dayton et al [19] have observed that the rate of drug disappearance (phenylbutazone, bicoumacetate, probenecid, and diphenylhydantoin) from dog plasma is dose dependent. Higher doses resulted in slower rates of metabolism than lower doses. The authors interpreted the results as dose dependent self-inhibition. Similarly, Thomas et al [20] have indicated that high doses of acetanilide inhibited its own metabolism to phenylhydroxylamine. The idea of drug self-inhibition, in addition to self-stimulation, is very provocative. However, more work is needed to assess both the validity of dose dependent self-inhibition of drug metabolism and its effect upon therapy and toxicity.

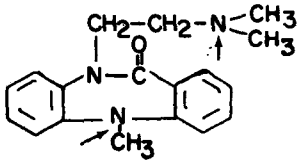
Application to Man - Buyske and Dvornik [4] have cited the use

of phenobarbital in treatment of hyperbilirubinemia as an example of the therapeutic application of enzyme induction. More information on this subject appeared during the past year. Crigler and Gold [21] reported that sodium phenobarbital lowered bilirubin concentration in an infant with congenital nonhemolytic jaundice and kernicterus by enhancing the rate of bilirubin excretion. Phenobarbital was also credited by De Leon et al [22] as being effective in human jaundice characterized by a relative deficiency of glucuronyl transferase, but not in the treatment of jaundice characterized by inability to form bilirubin glucuronide. This conclusion was based on studies in glucuronyl transferase deficient (Gunn) rats. Bakken and Fog [23] have postulated that unconjugated bilirubin may act as a trigger for its conjugation in man and have proven their hypothesis to be valid in the rat.

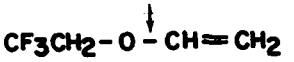
An additional item of therapeutic interest was presented by Solomon and Schrogie [24]. The authors have shown that the analgesic phenyramidol (Analexin) when given to subjects treated with diphenylhydantoin increases the mean biological half-life of the latter drug from 25 to 55 hours. Such inhibition of drug metabolism may result in elevated levels of diphenylhydantoin in plasma and could account for the observed increases in anticonvulsant activity and in possible side effects. The authors suggest avoiding concurrent administration of both drugs.

Metabolic Pathways of Drug Transformation - The following is a summary of drug metabolism from selected papers published in 1967. It will provide examples of general and some uncommon metabolic transformations of drugs. The latter will be pointed out in the text.

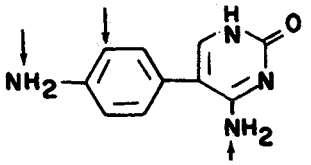
The summary includes chemical or generic names, structural formulae of the compounds, and their main therapeutic or pharmacological properties. The arrows indicate sites of the described metabolic transformations. The abbreviations used are: UP, unchanged parent compound; (M), major metabolite; (m), minor metabolite; (t), trace amounts; gluc., glucuronide conjugate; gly., glycine conjugate; sulf., sulfate conjugate.

1. 

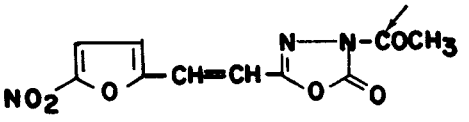
Noveril, antidepressant [25]. Man and dog: demethylation of side chain and ring N; ring hydroxylation (position unspecified) produces phenolic hydroxy compounds present as gluc.; UP in urine. Rabbits: demethylation (mono) of side chain and ring N, then formation of gluc. Metabolites found [26] in brain of rats, mice, rabbits.

2. 

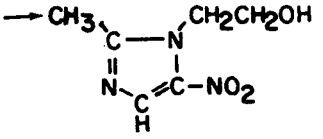
Fluroxene, volatile anesthetic [27]. Mice and dogs: formation of trifluoroethanol gluc., trifluoroacetic acid, and CO_2.

3. 

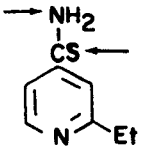
5-(4-Aminophenyl)-cytosine, antiviral [28]. Mouse: hydroxylation of aminophenyl (8%), then formation of gluc. (t); acetylation of aminophenyl (18%), then hydroxylation of acetamidophenyl product (6%); deamination of cytosine and hydroxylation of acetamidophenyl product (3%); UP (62%) in urine.

4. 

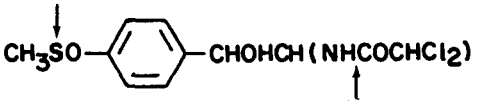
4-Acetyl-2-[2-(5-nitro-2-furyl)vinyl]-Δ^2-1,3,4-oxadiazoline-5-one, antimicrobial [29]. Man: one metabolite identified as deacetylated derivative (<1%) in urine. Metabolite more active and more toxic than parent.

5. 

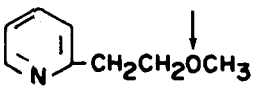
Metronidazole, trichomonacidal [30]. Man: oxidation of 2-methyl group to corresponding alcohol (M); UP in urine.

6. 

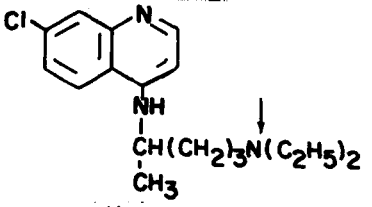
Ethionamide, antituberculosis [31]. Man, rats, mice, dogs: sulfoxidation then formation of ethylisonicotinamide which is deaminated. This represents a new pathway; UP in urine. Ethionamide sulfoxide [32]. Mouse and man: metabolism more rapid than that of ethionamide.

7. 

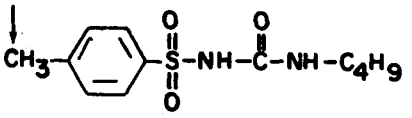
2,2-Dichloro-N-[β-hydroxy-α-(hydroxymethyl)-p-(methylsulfinyl)-phenethyl] acetamide, antibacterial [33]. Mouse: sulfoxide reduction. Rat and monkey: reduction and oxidation of sulfoxide. Dog: sulfoxide reduction, and deacetylation; UP in urine. Metabolites are active antibacterial agents.

8. 

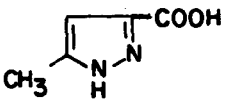
Methyridine, anthelmintic [34]. Sheep, calves, cows, rabbits, rats: O-demethylated to the corresponding primary alcohol; further oxidation to pyrid-2-ylacetic acid (82%) excreted unchanged or as gly. in urine.

9. 

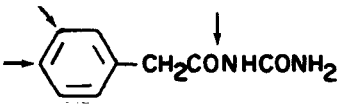
Chloroquine, antimalarial [35]. Man: N-deethylation to desethylchloroquine (23%), bidesethylchloroquine (2%); oxidative deamination to carboxylic acid (m); UP (70%) in urine. Chronic dosing (114 d.) did not affect own metabolism. Monkey [36]: oxidative deamination to carboxylic acid (M), and N-deethylation; UP in urine. Chronic treatment increases formation of desethyl metabolite.

10. 

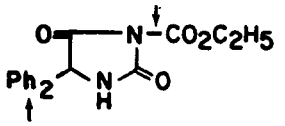
Tolbutamide, hypoglycemic [37]. Rat and rabbit: oxidation to hydroxymethyl product (M); carboxy derivative (m); UP in urine. Contrasted to early data identifying carboxy derivative as major metabolite.

11. 

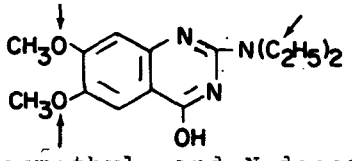
5-Methylpyrazole-3-carboxylic acid, hypoglycemic [38]. Man and rat: gly. (40%); UP (60%) in urine. In dog drug excreted unchanged.

12. 

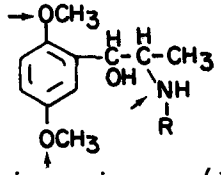
Phenacetylurea, anticonvulsant [39]. Rabbit: hydroxylation of benzene ring at C-3,4-followed by methylation of the 3-hydroxy derivative (12%); hydrolysis of ureide group then gly. (12%); UP (7%) in urine. Novel pathway - first example of successive hydroxylation and methylation of a drug in vivo.

13. 

3-Ethoxycarbonyl-5,5-diphenylhydantoin, anticonvulsant [40]. Rat: loss of ethoxycarbonyl group, to form active metabolite Dilantin. Dilantin then further metabolized via p-phenyl hydroxylation to inactive metabolite. Excretion of active and inactive metabolites into urine and feces. Low recovery of administered material indicated.

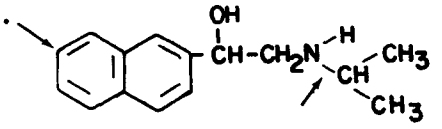
14. 

2-Diethylamino-6,7-dimethoxy-4(3H)-quinazolinone, hypotensive [41]. Man: O-demethylation and N-dealkylation. Five metabolites described representing mono and di O-desmethyl, and N-desethyl derivatives (<10%); UP in urine.

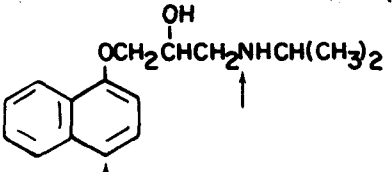
15. 

(I), R=CH(CH3)2
(II), R=H
(III), R= tC4H9

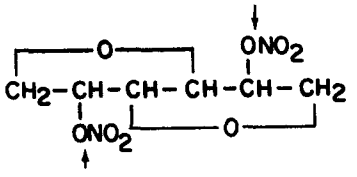
N-Isopropylmethoxamine (I), and Methoxamine (II), adrenergic blockers [42]. Rats, dogs: (I) O-dealkylation and conjugation (M), N-dealkylation (m); UP in urine. (II) O-deakylated in dogs (12%); UP (38%) in urine. No N-dealkylation of (III) in dog.

16. 

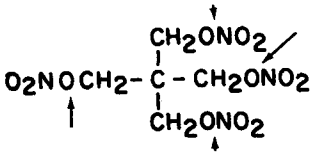
Pronethalol, adrenergic blocker [43]. Metabolized via 2 pathways: (a), N-dealkylation followed by oxidation, forming 2-naphthylglycollic acid (M) which is oxidatively decarboxylated (M); (b), ring hydroxylation at C-7 and gluc.; UP (t) in urine. Species difference in pathway. Ring hydroxylation and gluc. (M) in rabbit, cat, rat, monkey, man. Side chain oxidation (M) in guinea pig.

17. 

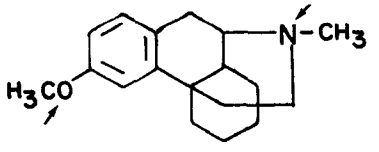
Propranolol, adrenergic blocker [44]. Rats, mice, rabbits, guinea pigs, man: side chain oxidation (m), propranolol gluc. (M), ring hydroxylation at C-4 and gluc. (M); UP (t) in urine.

18. 

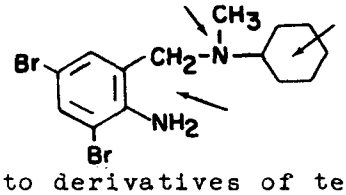
Isosorbide dinitrate, coronary vasodilator [45]. Dog, man: denitration (M); metabolites, isosorbide and its 2 and 5 mononitrates (t), in urine.

19. 

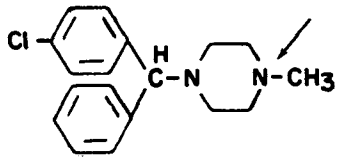
Pentaerythritol (PE) tetranitrate, antianginal [46]. Rat: denitrated, forming PE (M), PE-dinitrate (m), PE-mononitrate (t), in urine and feces.

20. 

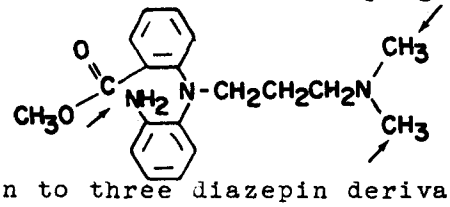
Dextromethorphan, antitussive [47]. Rat: O-demethylation and gluc. of 3-hydroxy derivative (M), complete demethylation then sulf. and gluc (m). Biliary excretion-major route for metabolites; UP (3%) and metabolites in urine.

21. 

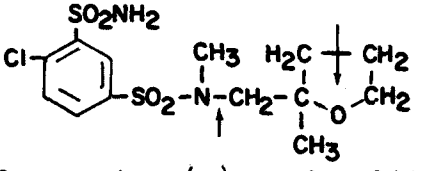
Bisolovon, stimulator of bronchial secretion [48]. Rabbits: extensive metabolism (12 products); hydroxylation of cyclohexyl ring (30%); N-demethylation (m); cyclization to derivatives of tetrahydroquinazoline (m); UP (30%) in urine. All metabolites gluc.

22. 

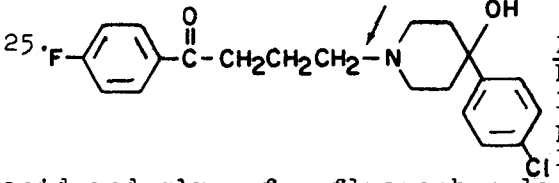
Chlorcyclizine, antihistamine [49]. Man and rat: N-oxide formation (m)- represents a new metabolite. N-oxide not converted directly to norchlorcyclizine in vitro. N-oxide demonstrated in pregnant rat urine [50].

23. 

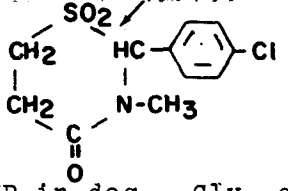
Methyl N-(O-aminophenyl)-N-(3-dimethylaminopropyl) anthranilate, diuretic [51]. Man and dog: N-demethylation (30%); intramolecular condensation to three diazepin derivatives (m); UP (4%) in urine. Decreased metabolism or change in excretory pathway occurred upon chronic dosing.

24. 

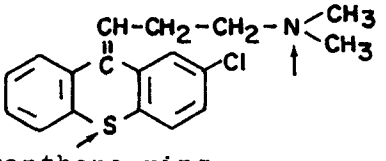
N-(4'Chloro-3'sulfamoylbenzene sulfonyl)-N-methyl-2-aminoethyl-2-methyltetrahydrofuran, diuretic [52]. Man and rats: drug almost completely metabolized; oxidation of furan ring (M), and split of molecule to 4-chloro-3-sulfamoyl-N-methyl-benzene-sulfonamide (m), and γ-carboxy-γ-valerolactone (m).

25. 

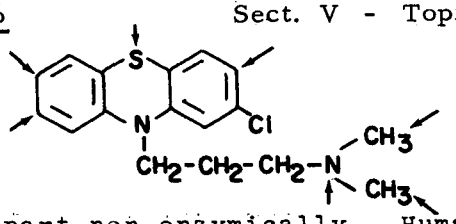
Haloperidol, neuroleptic [53,54]. Rat, dog: oxidative N-dealkylation to β-(p-fluorobenzyl)-propionic acid; further metabolized to p-fluorophenylacetic acid and gly. of p-fluorophenylaceturic acid (40%). Unknown polar metabolite (35%); UP (1%) in urine.

26. 

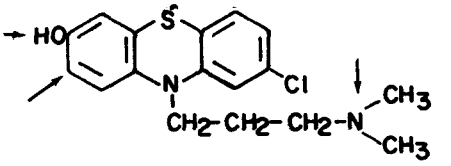
Chlormezanone, CNS depressant [55]. Man, dog: non-enzymic hydrolysis, to form 4-chlorobenzaldehyde then oxidation and gly. (M); UP (1%) in urine; biliary excretion of UP in dog. Gly. of chlorobenzoic acid represents a unique pathway since gluc. is the usual metabolite of benzoic acid.

27. 

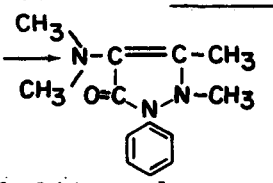
Chlorprothixine, tranquillizer [56]. Rats and dogs: sulfoxidation and N-demethylation (mono) of sulfoxide derivative; UP in urine. Dogs: hydroxylation of thiaxanthene ring.

28. 

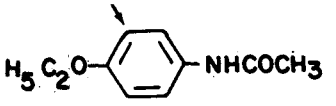
Chlorpromazine, tranquillizer. Rat liver microsomes, in vitro [57]. N-oxidation (M), previously described as minor metabolite; sulfoxide produced, in part, non-enzymically. Human liver microsomes, in vitro [58]: N-oxidation, N-oxide reduction, and ring hydroxylation, in addition to previously described pathways.

29. 

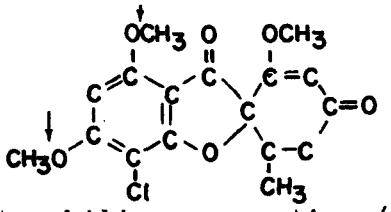
7-Hydroxychlorpromazine, (major metabolite of chlorpromazine) [59]. Rabbit liver microsomes, in vitro: (a) mono N-demethylation (M); (b) hydroxylation then O-methylation to form O-methylated 7,8-dihydroxychlorpromazine; this represents a new metabolic pathway.

30. 

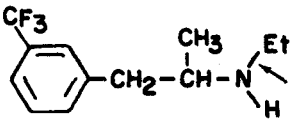
Aminopyrine, antipyretic, analgesic [60]. Cow: N-demethylation to aminoantipyrene (9%) which is acetylated (24%) or hydroxylated (.5%); UP (32%) and metabolites gly. or gluc. in urine. In contrast to dog where little acetylation occurs. Rabbit [61]: N-demethylation (M); in rat acetylation (M); metabolism indicated in rabbit foetus. UP and non-conjugated metabolites detected in cow milk [62].

31. 

Phenacetin, antipyretic, analgesic [63]. Rat: 3-hydroxyphenacetin, a new metabolite (m) detected in urine; metabolite also formed in vitro.

32. 

Griseofulvin (G), antifungal [64]. Rat: extensive biliary excretion and enterohepatic circulation; 4-demethyl-(G) in bile (M), 6-demethyl-(G) in bile (m). Rabbit: biliary excretion (m); 6-demethyl-(G) in urine (M). Metabolites, free and conjugated.

33. 

Fenfluramine, anorectic [65]. Man: N-deethylation (M); UP in urine. Low recovery of administered material indicated. Rate of excretion of metabolite and UP dependent on urinary pH.

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Chapter 23.
Immunochemical Mechanism of Drug Allergy

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Introductory Clinical Comments - The problem of drug allergy is one of considerable importance for the drug industry. The incidence of such adverse drug reactions varies with different drugs. For penicillin, allergic reactions occur in about 1-3% of patient courses. For other drugs, the incidence of allergic reactions is generally lower. However, nirvinol, a hydantoin derivative, which is no longer used, caused allergic reactions in 50-80% of patients treated with it. Some adverse drug reactions are clinically serious, and others such as anaphylaxis, can cause death. The many clinical syndromes believed to be allergic drug reactions are shown in Table I:

Adverse Reactions Associated with Drug Therapy Thought to be Allergic-in-Mechanism

Immediate (Occur within 1 to 30 minutes after initiation of drug therapy)
- Urticaria, diffuse flush, angioedema
- Hypotension, shock
- Asthma, laryngeal edema

Accelerated (occur 1 to 72 hours)
- Urticaria, angioedema
- Laryngeal edema, asthma

Late (Occur 3 days or longer after initiation of drug therapy)
- Urticaria, angioedema
- Exanthematic eruptions, purpuric eruptions
- Erythema multiforme, nodosum
- Exfoliative dermatitis
- Fixed drug eruptions
- Fever
- Serum-sickness like reactions
- Hemolytic anemia
- Thrombocytopenic, low-thrombocytopenic purpuras
- Agranulocytosis
- Hepatitis, cholestatic and non-cholestatic
- Nephritis
- Myocartitis
- Pulmonary reactions
- Systemic lupus erythematosis

Just as the drug industry has taken steps to decrease the toxicity of useful drugs, it might now study ways of predicting and decreasing allergenicity of drugs. A practical approach to this problem would be based on a rational understanding of the immunochemical mechanism involved in drug allergy.

Mechanisms of Drug Allergy-Overall View - This discussion will be confined to low molecular weight or "simple chemical" drugs. These are drugs of

well-defined organic chemical structure with molecular weights under 500 or at most under 1000. By drug allergy is meant those adverse drug reactions which are mediated by immunological mechanisms, i.e., by antigen-antibody reactions that occur in tissues. We are not considering idiosyncratic adverse drug reactions which are mediated by biochemical mechanisms, e.g. primaquine induced hemolytic anemia in individuals with genetically controlled deficiencies of glucose 6-phosphate dehydrogenase.

Three groups of unrelated molecular events are involved in the causation of allergic drug reactions. These are: 1. The formation of hapten-protein conjugates from the drug. These conjugates are the "complete immunogens" (i.e. substances which can induce antibody synthesis) responsible for inducing drug antibodies. This step may require drug metabolism and coupling of a reactive intermediate with tissue proteins. 2. The induction of antibody synthesis by the complete immunogens formed from the drug. Here, several different classes of antibodies, which can mediate different kinds of allergic reactions, may be induced. This is dependent upon genetic and environmental factors. 3. Antibody-antigen interaction in tissue will result in the formation of complexes. Certain antigen-antibody complexes can induce allergic tissue damage; others are relatively benign. Allergic tissue damage is generally mediated by pharmacological mediators (e.g. histamine) or by lytic enzymes which are released from tissues following antigen-antibody interaction.

A clinical allergic reaction thus results from the above sequence of physiological events. Interfering with any one event in this sequence would prevent or abbrogate the allergic drug reaction. This interference could constitute the pharmacological attack upon the problem of drug allergy.

The plan of the remainder of this exposition will be to discuss each of these 3 groups in more detail. Since much work has been done with penicillin allergy, this subject will be used frequently as a model. Where hard data are few, speculative hypotheses will be presented in the spirit of stimulating scientific dialogue. At the end will be presented recent work on the development of objective immunological tests to predict the potential allergic reactor to penicillin. This is meant to serve as an example of the practical application of the principles involved. The area to be covered is broad, including aspects of drug metabolism, immunology, and clinical allergy. Some oversimplifications are inevitable.

Immunogenicity of Simple Chemical Drugs, Irreversible Binding Theory - It is widely accepted among immunologists that in order for a simple chemical to induce an immune response, (i.e. induce the organism to synthesize specific antibodies) it must first bind irreversibly to tissue macromolecules, presumably proteins. According to this concept, the conjugated proteins formed from this union (termed hapten-protein conjugates) can induce the synthesis of antibodies specific for the drug, whereas the unconjugated drug itself is incapable of inducing antibody synthesis. This "irreversible binding theory" is based upon the empirical observations made by many investigators that of a large group of simple chemicals tested for

immunogenicity, only those which were capable of reacting irreversibly with proteins, or with protein-model compounds, could induce a strong immune response. Thus, Landsteiner & Jacobs[1] found initially that of 17 isomeric nitrochlorobenzenes, only the 10 which could bind covalently to aniline (and thus, by inference, with amino groups of proteins), could induce hypersensitivity in guinea pigs. Similar findings were later made by Gell et al[2] in guinea pigs and rabbits, and by Sultzberger et al[3] in man. These findings were extended by Eisen et al[4] who demonstrated that of a group of 2,4-dinitrophenyl (DNP) compounds, the sensitizers could react irreversibly with amino and sulfhydryl groups of epidermal proteins in vivo, whereas the nonsensitizers could not. Eisen observed a rough correlation between the chemical reactivities of a series of DNP compounds and their abilities to sensitized guinea pigs for anaphylaxis. In addition, preformed DNP-protein conjugates were found to be more potent sensitizers than the most highly reactive of the low molecular weight DNP compounds tested.[4] Finally, the classes of organic compounds which regularly induce hypersensitivity are all known to react irreversibly with proteins. These include acid anhydrides, acid chlorides, reactive aromatic halides, isocyanates, and isothiocyanates, mercaptans, quinones, oxazolones, and diazonium salts.

In immunogenic hapten-protein conjugates, the hapten is bound to the carrier protein almost invariably through covalent bonds. Covalent bonds are strong bonds with binding energies in the order of 50Kcal/mole. In rare instances immunogenic conjugates can result from the binding of prosthetic groupings to carrier proteinthrough multiple ionic bonds. For example, it was found that a mixture of oligonucleotides with methylated bovine serum albumin could induce, in rabbits, antibodies specific for the oligonucleotides, whereas the oligonucleotides alone were non-immunogenic.[5] The polyanionic oligonucleotides appear to bind to the polycationic methylated albumin through multiple electrostatic and hydrogen bonds. The summation of electrostatic and hydrogen bonding could provide as much as 10Kcal/mole (depending upon the dielectric constant of the medium) per bond. As few as 5 such bonds per mole could then provide as much binding energy as a covalent bond.

Simple reversible protein binding, such as sulfonamides to human sera albumin, does not appear to result in immunogenic conjugates. This has been shown in the case of dinitrophenol which binds relatively strongly but reversibly to albumin, but which cannot induce a DNP-specific immune response.[4] However,it is still possible that in very rare instances, multitonic drugs might be immunogenic through the formation of multiple noncovalent bonds with proteins.

The theoretical basis for the apparent obligation for irreversible binding is not known. This is due to our lack of knowledge of the basis for immunogenicity of macromolecular immunogens such as proteins. As to the latter, there are at present two major working hypotheses. One is that the immunogen must first be metabolized, presumably in macrophages.[6,7] According to this hypothesis, the biochemical processing of the immunogen results in the formation of "inducer" substances (apparently RNA,either coupled to antigenic fragments, or informational RNA) which can stimulate lymphoid

cells to proliferation, transformation to plasma cells among others, and antibody synthesis. The other hypothesis,[8] is that the immunogen must be trapped and retained in the intercellular spaces between membranes of a dendritic web of interdigitating reticular cell processes in the follicular structures of lymph nodes and spleen. There the immunogen may stimulate certain lymphoid cells to proliferation, transformation, and antibody synthesis. These hypotheses are admittedly vague, as this field is in its early stages of development. However, with either view, it may be considered that the simple chemical drug itself cannot undergo either the hypothetical necessary biochemical steps mentioned, or it cannot bind in the appropriate intercellular loci, which is necessary for immunogenicity. Strong binding to protein would then permit the simple chemical to utilize the biochemical pathways available to the protein, or to utilize the ability of proteins to localize and remain in the appropriate intercellular sites, which is necessary for immunogenicity. Strong binding, best exemplified by covalent binding, might be viewed as being needed for the hapten-protein conjugates to survive conditions which would essentially completely break weakly-bound hapten-protein conjugates.

This brief speculative discussion is presented in an attempt to rationalize the empirically derived obligatory binding hypothesis for the immunogenicity of simple chemicals. It serves also to indicate the current thinking on the mechanisms of immunogenicity. This is an area of considerable interest for the medicinal chemist. The placing of appropriate blocks in the pathway of antibody synthesis (particularly of specific antibody classes) would constitute a general approach to immunotherapy, not only of drug allergy, but of other immunologically mediated diseases as well as of tissue transplantation.

Role of Drug Metabolism and Degradation in Immunogenicity - Most allergenic drugs do not appear to be capable of covalent binding with proteins. For such drugs (e.g. sulfonamides, barbiturates, local anesthetics, etc.), it is reasonable to postulate that either contaminants or degradation or metabolic products may be the chemically reactive substances responsible for immunogenicity. By degradation is meant transformations which do not require enzymatic catalysis. By metabolism is meant transformations requiring enzymatic catalysis.

An example of the role of degradation is the formation of the minor haptenic determinants of penicillin hypersensitivity.[9-12] At least one of them appears to be formed from benzylpenicilloate, a hydrolysis product which forms readily from penicillin in neutral aqueous solution *in vitro*.

It has been suggested recently[13] that the immunogenicity of penicillin is due, in part, to trace contaminants, including polymers of penicillin in combination with its degradation products, and foreign proteins. However, similar studies in several other laboratories have failed to find protein impurities in penicillin, and the polymers (which were found) have to-date been found to be non-immunogenic in rabbits (unpublished data). This question is thus presently unsettled.

There is presently no well worked out example of a drug metabolite being an intermediate in the formation of hapten-protein conjugates from drugs. This is probably due to the fact that very little work has been done in this area. There is a considerable amount of suggestive evidence that drug metabolism may be involved in drug allergenicity for the majority of allergenic drugs. Much of this evidence points to the possibility that reactive oxidative metabolites may be involved in many cases, (e.g. quinones, quinonimines, free radicals, epoxides, etc.). The lines of evidence are as follows: 1. Initially, Landsteiner and Di Somma[14] found that guinea pigs could be sensitized to picric acid, and that such animals would cross react allergenically to picramic acid, a reduction product of picric acid. Since picric acid is not known to react chemically with proteins, and picramic acid could be readily oxidized to a quinone (which is protein reactive), the implication of this finding was that a quinone metabolite of picric acid might be responsible for its immunogenicity. Mayer's[15] extensive studies in guinea pigs showed a high degree of allergic cross-reactivity among aromatic nitro, nitroso, amino and phenolic compounds. He interpreted these results as suggesting that common quinone or quinonimines were formed metabolically and were responsible for the allergenicity of these aromatic compounds. 2. There is suggestive chemical evidence that certain aromatic compounds are converted to quinones when incubated with liver slices or homogenates in vitro: a. The metabolic conversion of nitro compounds to amines, and hydroxylation of aromatic amines and phenols to form o or p-aminophenols, or dihydroxyphenols is well known. The latter are readily oxidized by O_2 in mildly alkaline aqueous solution, non-enzymatically. In several cases this kind of oxidation has been shown to be markedly enhanced by the presence of rat kidney cortex mitrochondria and catalytic amounts of cytochrome C.[16] b. Riegel and Miller[17] showed that C^{14} tagged estradiol binds irreversibly to proteins of a fortified rat liver homogenate. They found that the presence of an electron donating substrate such as hexose-1, 5-diphosphate, and an electron transporting coenzyme such as TPN, and O_2 were necessary for irreversible protein binding of the estradiol tag. They suggested that the aromatic phenol ring of estradiol may be converted to a o-hydroxyphenol which could be readily oxidized to a quinone. Quinones are known to react readily with sulphydyl and amino groups of proteins under mild conditions.[18] The reaction appears to be complex, but the first steps appear to be simple addition reactions.[18] c. Studies on the metabolism of simple chemical carcinogens would suggest that other oxidative intermediates such as free radicals, peroxides, or epoxides may be reactive proimmunogens derived from drugs. Based upon initial studies of the Millers,[19] Hulten[20,21] investigated the irreversible binding of radio-tagged dimethylaminoazobenzene to proteins of rat liver microsome preparations. Here too, O_2 and TPNH were necessary for binding, indicating the formation of chemically reactive oxidative intermediates. Whether the intermediates were quinones, epoxides, peroxides, or free radicals could not be established, since these unstable materials could not be identified or isolated. Hulten[20,21] suggested also that the extent of protein conjugation by a chemical carcinogen in vivo is determined by the ability of the chemical to penetrate to the proper intracellular metabolic locus, its extent of metabolism here, and also by the competition for the reactive oxidative intermediates by sulfate and glucoronide conjugation system.

This concept appears to pertain also to the metabolic formation of hapten-protein conjugates from drugs. Maximal production of such conjugates would require the relative lack of competitive metabolic machinery (which would metabolize the drug in ways preventing the formation of chemically reactive intermediates), as well as the relative lack of competing systems which would drain off the reactive metabolites (e.g. as glucuronides). In this regard Shahidi[22] recently reported two sisters who metabolize phenacetin in an unusual way. These individuals metabolized phenacetin to 2-hydroxyphenacetin and 2-hydroxyphenetidin. It was suggested that the primary difference between these individuals and "normals" might be a relatively inadequate de-ethylation mechanism in these individuals. This would permit a relatively minor metabolic pathway, ortho-hydroxylation, to become relatively major. These patients developed toxic hemolysis to phenacetin, possibly due to the excessive metabolic production of hydroxyphenetidin (and or its quinoneimine oxidation products). The patients were not described as being allergic to phenactin. However, 2-hydroxyphenetidin was shown to be easily oxidizable (to quinone-imine derivatives) and this pathway might easily account for allergic reactions to phenacetin in other individuals

Thus it appears probable that chemically reactive oxidative metabolites of many allergenic drugs may be intermediates responsible for their allergenicity. It would accordingly appear desirable to carry out studies on the metabolism of allergenic drugs such as sulfonanides, salicylate derivatives, etc., from the point of view of the formation of hapten-protein conjugates. In vitro systems utilizing radio-tagged drugs and liver slices or fortified liver homogenates may prove of value here as they have in the field of chemical carcinogenesis.

Immunogenicity of Benzylpenicillin - Considerable experimental work has demonstrated that the immunogenicity of penicillin is due to its ability to react chemically with tissue proteins to form several different haptenic groups.[9-12] The major haptenic determinant is the benzylpenicilloyl (BPO) group. It is termed "major" from the indication that of the quantity of penicillin which reacts chemically with protein, the largest percentage appears to bind as benzylpenicilloyl (BPO) groups. The first indication that the BPO group is an important determinant of penicillin hypersensitivity was obtained from immunological and organic chemical considerations. It was found that guinea pigs made contact allergic to penicillin cross-reacted completely to benzylpenicillenic acid (BPE), a rearrangement product of pencillin. Since BPE was found to react readily with amino groups of simple protein model compounds under physiological conditions to form benzylpenicilloyl-amines[23] the BPO group appeared a likely possibility. Firm proof for the BPO group was obtained by immunizing rabbits with benzylpenicillin and showing that the sera from these animals could be precipitated specifically by multivalent BPO-protein conjugates, and that the precipitation could be completely inhibited by structurally defined univalent benzylpenicilloyl amine haptens.[24] Similarly, some patients hypersensitive to penicillin, gave positive immediate skin tests to BPO-protein conjugates. These tests were inhibited by univalent BPO haptens.[24] BPO specificity of penicillin hypersensitivity has been amply confirmed.[9-12]

It is not yet clear whether the BPO group is formed by the direct reaction of penicillin with amino groups of protein, or through the intermediate formation of benzylpenicillenic acid. This question has been amply discussed elsewhere.[9-12,25] If formed directly from penicillin, the BPO group would be formed as α- diastereoisomer, as the asymetric carbons retain their configurations. The α-isomer then would mutarotate for form a diasterioisomeric mixture, as BPO-specificity is toward the diastereoisomeric mixture rather than toward the α-diastereroisomer alone.

The presence of minor haptenic determinants of penicillin hypersensitivity is inferred from immunological data. The structures of the determinants have not been defined chemically as yet. In guinea pigs made contact hypersensitive to penicillin, weaker reactions can be elicited by D-penicillamine and by benzylpenicilloic acid.[26] From considerations of the chemical reactivity of these compounds,[27] it is likely that mixed disulfide residues of benzylpenamaldic acid and D-penicillamine function as minor determinants. Similarly, certain patients hypersensitive to penicillin give immediate skin tests to benzylpenicilloic acid but not to penicillamine. These findings suggest that the benzylpenamaldic acid mixed disulfide group may be one of the minor determinants.[28] Other patients give positive skin tests to crystalline penicillin, but not to benzylpenicilloic acid, nor to multivalent BPO conjugates. Such patients thus produce antibodies to a haptenic determinant formed from penicillin which is not the BPO group nor is it a group formed from benzylpenicilloic acid.[28] The identity of this minor hapten determinant is not known. In addition to these two haptens, other possible minor haptenic determinants of penicillin hypersensitivity have been proposed.[10]

Genetic and Environmental Controls of Drug Immunogenicity - As genetic differences among individuals with regard to metabolic enzymes exist, this factor may determine, in part, which individuals in a population-at-risk will develop an allergic reaction to a drug. For example, it is conceivable that only a certain percentage of the population can metabolize a drug in the ways required for the formation of a chemically reactive intermediate, and for its conjugation with proteins to yield immunogenic hapten-protein conjugates. Shahidi's study[22] on the unique metabolic formation of large amounts of 2-hydroxyphenetidin from phenacetin is an example of this possibility. Environmental factors may also control drug metabolism. For example, phenobarbital can induce the synthesis of microsomal liver enzymes capable of enhancing hydroxylation of other drugs.[29] Thus in Shahidi's study pretreatment of the patient with phenobarbital resulted in an enhancement of her toxic reaction to phenacetin, presumably due to an enhanced production of hydroxyphenetidin.[22] Thus genetic and environmental factors may influence drug metabolism the production of hapten-protein conjugates and determine, in part, which individuals develop drug allergy. However, other environmental and genetic factors influencing drug allergy may operate at the level of the immune response and at subsequent levels.

Antibody Structure and Function - It is pertinent to review briefly the biological functions of antibodies, and how they mediate allergic

reactions. Detailed information can be found in several excellent reviews.[30-32] Antibodies are γ and β globulins, termed immunoglobulins. Antibody molecules are made up of two kinds of polypeptide chains, L or light chains (MW 25,000) and H or heavy chains (MW 50,000). The immunoglobulin G (IgG) molecule is made up of 2L and 2H chains held together by disulfide and non-covalent bonds (MW 150,000). Of importance to the mechanism of allergic diseases, immunization of man with a given antigenic determinant may induce the synthesis of antibody molecules of several different antibody classes. There are 5 classes presently known for man. These different antibody classes are similar in that they are all immunologically specific for the antigen which induced their synthesis, and they have the same or similar L-chains. They differ in structural configurations of the H chain, and in their biological properties related to the different H chain. For example, whereas all antibody classes can bind antigen, only some can fix complement and mediate allergic reactions that require complement fixation, e.g. Arthus reactions and experimental serum sickness due to antigen-antibody complexes. Thus the antibody classes which are stimulated by a given drug will determine, in large part, whether the patient will have an allergic reaction and the form it will take.

There are 5 immunoglobulin (Ig) classes recognized in man at the present time (IgG, IgM, IgA, IgD, and IgE) and there may be more. These classes are differentiated on the basis of their H chains. In addition there is delayed hypersensitivity, which is, at present, believed to be mediated by sensitized lymphoid cells and not be circulating antibody molecules. IgG is the common 7S (150,000 MW) antibody which, for example, is produced in large quantity by the rabbit. IgM is the macroglobulin antibody (MW 1,000,000). Both these classes can fix complement and, for example, can mediate the immunological destruction of blood cells in man in the presence of antigen (i.e., hemolytic anemia, thrombocytopenia, granulocytopenia). IgA has the unique function of being secreted in external secretions, e.g. saliva, intestinal fluid, etc., and is believed to play an important role in immunity against viral diseases. It has no known unique function in immunopathology as yet. IgD has no known unique function in immunopathology. IgE is a recently described immunoglobulin[33] which carries the reagins (also called skin sensitizing antibody, anaphylactic antibody or homocytotrophic antibody). This antibody has H chain configurations which permit its firm fixation to certain cell membranes (presumably mast cell membranes, based upon studies in rat models). Bridging of these "fixed" IgE antibody molecules with antigen, stimulates mast cell degranulation and liberation of histamine and other mediators, thus causing anaphylaxis. However it is not rigorously proved that histamine release in man requires degranulation. The IgE antibody is an important mediator of anaphylactic and urticarial drug reactions in man as well as allergic rhinitis and asthma, i.e., the atopic diseases of man. Since the incidence of atopy in the general population is about 15%, it is potentially an important area for the design of drugs. The attack upon atopy may be based upon specific interference in the production of IgE, in its fixation, in the histamine release process, as well as the well-known development of antimediators.

Antibody Assays - Reagins (IgE) are assayed by direct immediate skin tests for wheal-and-flare reaction, and by the Prausnitz-Kustner (P-K) test.[34] The former appears to detect skin-fixed reagins, the latter circulating reagins. The direct skin test is the more sensitive test. Reagins can be assayed also by in vitro techniques using human peripheral leukocytes[35] or monkey ileum[36] or chopped lung. These in vitro methods are currently less sensitive than the P-K test, or not completely specific for IgE.

The optimal antigenic reagent for the detection of reagins appears to be a trivalent hapten where the haptenic groups are separated by optimal distances, approx. 15-20 A. This information is based on studies in the benzylpenicilloyl system in man.[37]

Assays for Serum Antibodies - Serum antibodies appear to be present in rather low concentrations in drug hypersensitivity in man. For example, in penicillin hypersensitivity, the serum concentrations of BPO-specific antibody protein is under 20-30 μg per ml. and most commonly under 5 μg/ml. Thus the quantitative precipitin analysis, which cannot accurately assay less than about 50 μg/ml, cannot generally be used for drug hypersensitivity. However,there are rare reports of precipitating antibody in drug allergy.

The passive hemagglutination method is very sensitive and has been used to assay drug-specific antibodies in man. In penicillin hypersensitivity, the method[38] consists of reaction of human group O red blood cells (RBC) with penicillin at pH 9.8, which couples BPO groups to RBC surfaces. Serial serum dilutions are then incubated for 1 hour with aliquots of the BPO-coupled RBC. The antibody concentration is expressed as a titer, i.e., the highest dilution which gives detectable agglutination of BPO-RBC. This method detects BPO-specific antibodies. This specificity is ascertained by specific inhibition of hemaglutination with a univalent BPO hapten, BPO-n-propylamine. This method is very sensitive. It can detect as little as 0.0005 μg/ml of rabbit anti-BPO antibodies, or less than 10^{-12} molar concentration of these antibodies. IgM and IgG can be distinguished by noting the effect of treatment of the serum with 0.1 M mercaptoethanol (ME) upon the titer. Generally speaking, IgM hemagglutination activity is completely destroyed by ME, whereas IgG activity is not affected. However where IgG predominates, lower titers of IgM which may coexist in the serum would escape detection by this method. Similarly the lower titers of IgA, IgD,and IgE antibodies would also escape detection in the presence of comparatively large amounts of IgG in any serial dilution method.

The passive hemagglutination method is probably applicable to other drug allergies once the haptenic determinants are known. Other sensitive methods such as radioimmunoelectrophoresis and specific neutralization of hapten-coupled bacteriophage will probably be useful also. The use of drug induced blast transformation and DNA-synthesis in peripheral leukocytes for the assay of drug hypersensitivity has been reported, but it is not yet known what immune factors are actually being measured nor is its clinical meaning yet clear.

Immune Responses to Penicillin - The passive hemagglutination system to assay IgG and IgM antibodies of BPO specificity as well as direct skin testing to assay reagins were used. Skin tests were done with benzylpenicilloylpolylysine (BPL) to detect BPO-specific reagins, and a minor determinant mixture (MDM) to detect minor-determinant specific reagins. The MDM contains penicillin, benzylpenicilloate, benzylpenilloate and benzylpenicilloylamine. These simple chemicals are presumed to elicit the minor-determinant test by reacting with soluble proteins in the skin test site to form the proper multivalent hapten elicitors. Although MDM is apparently an effective test reagent, little is clearly known about how it elicits positive skin tests.

A brief summary of the immune responses to penicillin[28] follows:

Virtually all patients tested had detectable antibody. Most had only IgM antibodies in low titers. After a course of penicillin, there was a moderate increase in the titer of IgM antibodies, a higher frequency of patients with IgG antibodies (35%), and a higher frequency of patients with reagins (9%). What distinguished the non-reactors from patients having anaphylactic or urticarial reactions was the presence of reagins among the reactors (100% vs 9%). Other kinds of allergic reactions were associated with other kinds of immune responses. Delayed hypersensitivity to penicillin is also found.[39] It is detected by skin testing with 0.1 ml of 0.1 M penicillin injected intradermally, and reading the test at 24-48 hrs. A positive test is an area of erythema and infiltration. It is clear that the mere presence of antibodies does not mean that a patient is allergic to penicillin. What is important in this regard is the class and concentration of the antibodies.

Diagnosis and Prediction of Penicillin Allergy - Associations between unusual immune responses to pencillin and the occurrence of allergic reactions permit inferences as to the immunological mechanisms involved. Urticarial eruptions (hives) as well as anaphylaxis (sudden shock-like states and acute obstruction to respiration) were associated with reagins. They are apparently mediated by this class of antibodies. Diagnosis of such reactions is supported by positive skin tests (for reagins). In some cases, morbilliform eruptions (red, measles-like rashes) are associated with high titers of IgM antibodies, and can be so diagnosed. Hemolytic reactions (anemia caused by rapid destruction of red blood cells) are apparently mediated by IgG antibodies.[40] Accordingly these immunological tests are helpful in the diagnosis of these kinds of drug allergy.

Prediction of the anaphylactic reaction to penicillin can be done by skin testing. Anaphylactic reactions appear to be mediated by reagins. Thus patients liable to an anaphylactic reaction to penicillin should be the ones with positive skin tests to BPL or the MDM. Clinical studies are bearing this out.[41] Of 200 patients with past histories of penicillin allergy, 170 had negative skin tests and 30 had positive tests. The 170 skin-test negative patients tolerated penicillin without anaphylactic or urticarial allergic reactions. Of 9 skin test positive patients given penicillin by gradual administration, 7 had urticarial reactions (which

were controlled with drugs). these tests are thus very promising as simple, objective clinical tests for serious penicillin allergy.

Summary and Final Comments - The mechanism of drug allergy involves a sequence of 3 groups of unrelated molecular events: 1. The formation of hapten-protein conjugates form the drug. This appears to require the formation of chemically reactive metabolites in many cases. 2. The induction of antibody synthesis. A given antigen may induce different antibody classes in different individuals. Different antibody classes have different biological functions and mediate different kinds of allergic reactions. 3. The mediation of allergic tissue injury by the antigen-antibody binding occurring in tissue. Here non-specific factors are frequently involved (e.g., complement), and tissue injury is frequently the result of the liberation of pharmacological mediators.

Theoretically, allergic drug reactions (and allergic diseases in general) can be prevented or abbrogated by interferring with this sequence at any level. Of classical interest to the medicinal chemist is the possibility of altering the fundamental immunogenicity of drugs by structural alterations. Considering the diversity of metabolic operations on drugs, it may be impossible to completely prevent drug immunogenicity. However, it appears realistic to suspect that appropriate molecular alterations might greatly reduce immunogenicity of drugs and the incidence of allergic reactions to simple chemical drugs.

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Chapter 24. Drugs Affecting Enzyme Systems

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Drugs which affect enzyme systems continue to play an increasingly important part of contemporary pharmacodynamics. During the past two years this has been evidenced not only by the many research articles in this field, but also by the reviews on the biochemical aspects of pharmacology,[1, 2] as well as on specific enzymes and their inhibitors.[3, 4] The following discussion will attempt to cover advances which have occurred during 1966-1967 in specific areas of current interest and which have not already been reviewed. These areas include drugs affecting (1) catecholamine metabolism, (2) monoamine oxidase, (3) 5-hydroxytryptamine metabolism, (4) histamine metabolism, (5) acetylcholine metabolism. Areas such as drug metabolism, protein synthesis, metabolic actions of catecholamines and the actions of steroids will not be covered since recent reviews on these subjects are covered in other chapters and sources.

DRUGS AFFECTING CATECHOLAMINE METABOLISM - Studies on catecholamine metabolism continue to dominate a major part of biochemical pharmacology. There are several reasons for this popularity, among them being the elucidation of the biochemical pathways of catecholamine synthesis and degradation, and the development of new drugs affecting them.

Tyrosine hydroxylase inhibitors - Perhaps the greatest effort is being directed toward studies of the enzyme tyrosine hydroxylase which represents the rate limiting step in catecholamine synthesis. This enzyme catalyzes the conversion of tyrosine to DOPA and is localized in the particulate fraction of the cell sedimenting at 16,000 x g. Inhibition of this enzyme has been found to be the most effective means of blocking the formation of norepinephrine.[5] Much of the biochemistry of this and other enzymes associated with catecholamines was discussed at the Second Symposium on Catecholamines and published in 1966.[6] Hundreds of compounds have been examined for anti-tyrosine hydroxylase activity, but only a few exhibited inhibitor activity *in vitro*, and these were mainly analogues of tyrosine or its catechol metabolites.[7] It has become apparent, however, that there is not always a relationship between *in vitro* and *in vivo* activity.[8] Of the many compounds thus far tested, two which have shown *in vivo* activity are α-methyl-l-tyrosine (α-MT) and H44/68 (the methyl ester-HCl of α-MT), both acting as competitive antimetabolites of tyrosine. Their mechanism of lowering tissue levels of norepinephrine is not related to an interference of the uptake, storage or release of catecholamines.[9, 10] The administration of α-MT results in a depletion of norepinephrine in various tissues of animals[11] and a reduction in the synthesis of catecholamines by 70 percent in man.[12] Although only the l-isomer of α-MT is active as a tyrosine hydroxylase inhibitor, the d-form potentiates the action of α-MT, a phenomenon which has been postulated to occur as a result of an effect on membrane permeability.[13] α-MT has served as a valuable experimental tool wherever the involvement of catecholamines has

been questioned. For example, the fact that α-MT selectively depletes catecholamines but not 5-hydroxytryptamine has been useful in determining the possible roles of these amines in the actions of reserpine. The fact that α-MT (but not p-chlorophenylalanine, a 5-hydroxytryptamine depleter through inhibition of tryptophan hydroxylase[14]) produced behavioral depression which exhibited a time course similar to the catecholamine depletion in brain indicates a possible causal relationship.[15] The ability of α-MT to antagonize the central actions of amphetamine also provides strong evidence that this latter compound exerts its stimulatory effect by a mechanism dependent upon endogenous biosynthesis of catecholamines.[16-18] A few words of caution need to be interjected here, for α-MT itself is able to produce a depressant condition when given in large doses. Moore et al[19] emphasize the toxic actions of 200-300 mg/kg doses of α-MT in single injections which result in a high frequency of deaths. Lethality is preceded by a state of depression, lethargy and hypothermia in rats associated with blood and organ abnormalities.

Other workers have compared α-MT with related compounds such as H44/69 (methyl ester of α-methyl-dl-tyrosine) and H59/64 (3, α-di-methyl-tyrosine methyl ester-HCl). All of these agents act similarly in blocking tyrosine hydroxylase, lowering tissue levels of catecholamines and producing a decrease in conditioned avoidance responses in mice and rats.[20] They have also been employed to distinguish between the "depleter type" and the "releaser type" agents.[21] It is evident that these two types of agents, while lowering tissue catecholamine levels, have vastly different characteristics and mechanisms.

Other interesting and significant findings regarding drugs affecting tyrosine hydroxylase include those of Levitt et al[22] who described a series of phenylcarbonyl agents as in vivo inhibitors of the enzyme. McGeer et al[23] found a group of 5-halogenated tryptophans to be highly effective as tyrosine hydroxylase inhibitors. dl-5-iodotryptophan produced a 50 percent inhibition at 12.5 x 10^{-7} M of rat brain homogenate activity. Burkard et al[24] reported on the unexpected finding of a considerable activation of tyrosine hydroxylase by the tranquilizer chlorpromazine. Nyback et al[25] also observed an increase in the accumulation of C^{14}-dopamine after administration of C^{14}-tyrosine in chlorpromazine-pretreated rats.

Amino acid decarboxylase inhibitors - The second step in catecholamine formation involves the decarboxylation of DOPA by an enzyme amino acid decarboxylase. Inhibitors of this enzyme are known, but because of the difficulty in blocking adequately catecholamine synthesis by this means, little has appeared on newer inhibitors. A group of hydroxamic acid derivatives was shown to be effective as inhibitors of amino acid decarboxylase and in lowering catecholamine levels in brain.[26] Creveling et al[27] employed a new in vivo method for determining amino acid decarboxylase and studied a variety of compounds as inhibitors. Several of the hydrazine compounds proved to be highly effective inhibitors. Some of the previously reported amino acid decarboxylase inhibitors of the hydrazide (Ro-4-4602) and the benzyloxyamine types (NSD 1055 and 1024) have been employed as

experimental tools in the study of decarboxylation by the enzyme and in the formation of catecholamines.[28-30]

An interesting sideline regarding drugs affecting this enzyme is the finding that phenobarbital produced an activation of amino acid decarboxylase[31] much like it activates the drug detoxifying microsomal enzymes. This activation was also blocked by the compound SKF 525A.

Dopamine β-hydroxylase inhibitors - The conversion of dopamine to norepinephrine depends on dopamine β-hydroxylase, a copper dependent enzyme which is inhibited both *in vitro* and *in vivo* by disulfiram.[32] The mechanism of inhibition by disulfiram is related to its reduction to form diethyldithiocarbamate, a copper chelating agent. The compound phenylethyldithiocarbamate also acts in this manner.[33] The administration of disulfiram causes a marked lowering of tissue norepinephrine and a corresponding increase in dopamine levels.[34, 35] The drug has also been employed to demonstrate whether certain pharmacological phenomena are dependent upon the β-hydroxylation process to form norepinephrine or related compounds, such as on the restoration of tyramine action in reserpinized animals,[36, 37] the replacement of norepinephrine by dopamine,[38] and to demonstrate the actions of amphetamine.[39]

MONOAMINE OXIDASE INHIBITORS - Inhibitors of the enzyme MAO still attract much attention of investigators in the area of the biochemistry and pharmacology of biogenic amines. New compounds have been uncovered as MAO inhibitors, among these including a number of aziridine derivatives,[40] iodinated phenols,[41] various carboline compounds,[42] and certain stryryl-quinoliniums.[43] Some of the latter two classes of compounds were of high potency *in vitro*, showing ID_{50}'s at concentrations below 10^{-7} and 10^{-8} M. A number of nitrofuran derivatives has also been studied extensively as MAO inhibitors. These agents are known as antiseptics, but in the intact animal some of them, such as furazolidone and nitrofurantoin, are biotransformed to MAO inhibitors of the hydrazine type.[44] Stern *et al*[45] suggest that 2-hydroxyethylhydrazine may be the active MAO inhibitor from furazolidone. The bioactivation of the nitrofuran compounds appear to be a function of the liver microsomal enzymes, analogous to the bioactivation and subsequent inactivation of modaline sulfate (2-methyl-3-piperidinopyrazine), another irreversible MAO inhibitor.[46] A variety of phenylalkylhydrazines have been tested for their MAO inhibitor activity by several pharmacological tests, and these have led to the finding of other effects which are not always found in the standard direct determinations of MAO inhibition.[47] The compound SU 11739 (N-methyl-N-2-propynyl-1-indanamine), a non-hydrazine MAO inhibitor which resembles pargyline in chemical structure, was reported to be some 15-25 times more potent *in vivo* as a MAO inhibitor.[48] It also displayed a considerably greater effect on brain MAO than on the liver enzyme *in vivo*. 4-Chloroamphetamine was also found to be a potent inhibitor of brain MAO *in vitro*, and, based on brain concentrations of the compound after its administration, Fuller[49] has postulated it as an *in vivo* MAO inhibitor as well. Finally, the observation of Ogawa *et al*[50] that glyeryl trinitrate (nitroglycerin) was an effective competitive inhibitor of MAO deserves attention, especially since MAO inhibitors have been considered as anti-anginal agents.

The role of MAO inhibition in the mechanism of adrenergic nerve blocking action of bretylium, debrisoquin, and other agents was investigated by Giachetti and Shore.[51] Spencer[52] also observed the adrenergic blockade by iproniazid and discussed the possibility of an action on the adrenergic terminals. However, others attribute a central vasomotor depressant as the mechanism of the antihypertensive effect of the MAO inhibitors.[53]

An important development that has emerged from the use of MAO inhibitors has been the various interactions that occur between these agents and a variety of substances. The interaction between MAO inhibitors and various foods is still of clinical concern, and the pharmacology of these paradoxical phenomena has been studied by Blackwell and his associates.[54] Aside from foodstuffs, MAO inhibitors also potentiate the duration of hyperpyrexia produced by pyrogens,[55] initially decrease then increase the toxicity of cholinergic agents in reserpinized mice,[56] potentiate the cardiovascular effects of sympathomimetic amines,[57] although amphetamine and related compounds protect against the irreversible MAO inhibitors.[58] The hypoglycemic action of insulin is also enhanced,[59] while the development of experimental diabetes by alloxan is prevented.[60] In certain instances the interactions are peculiar to specific MAO inhibitors, such as phenelzine causing an increase in brain γ-aminobutyric acid (GABA) levels, while tranylcypromine is devoid of this action.[61] The GABA effect of certain inhibitors is of interest since they act as anticonvulsants against metrazol shock.[62] The sympathomimetic cardiovascular action of phenelzine, but not of pheniprazine, exhibited tachyphylaxis more rapidly in the nialamide-pretreated cat.[63] This and other related evidence was considered by the authors that phenelzine could serve both as a substrate and inhibitor of MAO. The positive inotropic action of strophanthin-g on the dog heart may be potentiated or reduced by several MAO inhibitors, depending upon the mode of administration.[64] The extent of MAO inhibition, rather than the elevation of cardiac catecholamines, has been considered more important in the potentiation phenomenon. Some of the cardioactive glycosides, such as digitoxin, produced a dose-dependent inhibition of brain MAO.[65] This effect, however, does not appear to be related to their cardiotonic activity since strophanthin-g was not active in inhibiting MAO. The role of cardiac MAO is further clouded by the finding that phenelzine and several other MAO inhibitors are capable of partially blocking the development of myocardiopathy produced by large doses of isoproterenol.[66]

TRYPTOPHAN HYDROXYLASE INHIBITORS - The rate limiting step in the synthesis of 5-hydroxytryptamine is the hydroxylation of tryptophan which forms 5-hydroxytryptophan. Studies of this system and its inhibitors have recently been reviewed.[67-70] Levi and Green[67] reviewed through 1966, so most references here will be of 1967 only. Since the discovery of p-chlorophenylalanine (p-ChPhe) as an inhibitor of tryptophan hydroxylase[14] most investigations are involved with this compound and its analogues such as α-methyl-p-chlorophenylalanine.[71] p-ChPhe is a competitive inhibitor of tryptophan-hydroxylase *in vitro* but is an irreversible inhibitor *in vivo*.[72] Inhibition of tryptophan hydroxylase occurs in rat and rabbit brain stem,[73]

in beef, rat[73] and human[74] pineal gland, and human carcinoid tumor.[73] In patients with carcinoid syndrome, p-ChPhe was found to lessen the gastrointestinal symptoms.[75] Several behavioral studies have been done with animals depleted of 5-hydroxytryptamine with p-ChPhe.[76, 77] There is a small but consistent decrease of norepinephrine in the brains of mice from p-ChPhe.[78] p-ChPhe has been used as a model of phenylketonuria in rats. Amino acid imbalances similar to phenylketonuria occurred, but 5-hydroxytryptamine levels were much lower than that normally occurring in patients.[79] Tryptophan and phenylalanine hydroxylase are also inhibited by esculetin and H22/54[80] and a variety of catechol compounds.[81] The other enzymes involved with synthesis (amino acid decarboxylase) and degradation (monoamine oxidase) are so parallel to the catecholamines that they are covered in their respective sections.

HISTIDINE DECARBOXYLASE INHIBITORS - The subject of histamine has regained considerable interest among chemists and biologists as evidenced by several recent reviews.[82, 83] Of special relevance is the biosynthetic step via histidine decarboxylase, the enzyme decarboxylating l-histidine to form histamine. Shepherd and Mackay[83] wrote an excellent review of this topic in 1967; therefore, no attempt will be made here to discuss the properties of the enzyme system.

Several inhibitors of histidine decarboxylase have been investigated extensively by Johnston and Kahlson[84] and by Levine, including the action of NSD 1055 (4-bromo-3-hydroxybenzyloxyamine) in humans. Levine and Watts[85] also reported on the inhibition of histidine decarboxylase activity in vitro by norepinephrine and related compounds. This is in contrast to the work by Pearlman and Waton[86] who demonstrated that injections of epinephrine into mice increased histamine formation by skin, lung and skeletal muscle, but not by stomach tissue. Apparently the source of histidine decarboxylase is of great importance as to the effects of inhibitors or potentiators.

A number of acidic anti-inflammatory agents, such as salicylate, phenylbutazone and indomethacin, have been considered as histidine decarboxylase inhibitors.[87] Their ability to inhibit histamine formation appears to be related to a competition with pyridoxal phosphate for the coenzyme binding site.

ACETYLCHOLINESTERASE INHIBITORS - The acetylcholinesterase inhibitors have been used as pharmacological tools in the study of cholinergic mechanism in the automatic nervous and neuromuscular systems. They are used in various disease states, such as myasthenia gravis. Agricultural use of esterase inhibitors is extensive, but because of space limitation the research on this interesting area is not reviewed here. There are two major classes of inhibitors, the carbamates and the organophosphates. These two groups will be reviewed with respect to their mechanism of action, chemical interactions and pharmacology in the mammalian system.

Carbamates - Structure activity relationships have been studied with respect to toxicity and anticholinesterase activity.[88, 89] The mechanism of acetylcholinesterase inhibition by the carbamates is not entirely clear.

Although kinetic studies of inhibition by monomethylcarbamates indicate these compounds to be poor substrates[90] there is evidence that carbamylation[91] and the actual structure of the "leaving group" is of most importance.[92] That carbamylation does occur is supported by the destruction of eserine and sevin[93] and by carbamylation rate constants.[94]

Organophosphates - As with the carbamates, studies of the mechanism of action and elimination have been performed. Kinetic studies indicate that dealkylation in a series of organophosphates is a first order rate.[95] The inhibitory action of diisopropylfluorophosphate (DFP) was found to be related to its affinity toward the enzyme; both inhibition and affinity greater for serum cholinesterase than for erythrocyte acetylcholinesterase.[96] The effect of substituent groups on phosphate esterase has been kinetically determined and electronic, steric and hydrophobic factors are implicated in their action.[97] Studies indicate that pH levels above 8.5 decrease inhibitory actions of phosphate esters and carbamates.[98]

Detoxification of organophosphates indicates that in low doses malathion will inhibit esterases which normally destroy it before inhibition of cholinesterases,[99] although it is not a substrate of serum cholinesterase.[100] Sarinase, the enzyme which hydrolyzes sarin, is found to be less effective toward l-sarin than d-sarin.[101] The microsomal detoxification of parathion has been shown to be accomplished by desulfonation.[102]

Reactivation - Antidotal actions toward compounds with the relative potency of the organophosphate insecticides is of continued interest. The general mechanism of reactivation of organophosphates has been reviewed[103] and there is continued research for new reactivators and comparison with those existing.[104, 105] Reactivation by oximes of acetylcholinesterase inhibited by DFP would be expected to cause decrease of DFP in the tissue, but this is not the case. The amount of DFP bound in tissue is not influenced by either oximes or atropine.[106] The aging process in which the enzyme becomes unreactivatible has been studied in dog with respect to the half-time aging for soman and was found to be 5.3 minutes *in vitro* and 5.5 minutes *in vivo*.[107] The actual process of aging of sarin has been determined to be a 1:1 stoichiometric relationship between the isopropyl alcohol and nonreactivatible esterase,[108] and the percent of enzyme not reactivated by oxime approximated the amount of methylphosphonate as measured isotopically.[109] Other factors involved with aging are the nature of the alkyl group and temperature changes.[110] In general, aging of plasma cholinesterase and acetylcholinesterase is the same with respect to bond fission.[111]

In vitro studies of reactivation of phosphorylated serum cholinesterase indicate a varying effect of salt concentration on the reactivation process.[112] The prophylactic use of oximes has been suggested, and the half-life of 2-PAM was determined to be 1.4 hours.[113] The half-life of the oximes TMB-4 and toxogonin has been determined to be 28.3 and 19.9 minutes, respectively.[114]

Other agents affecting cholinesterases - By changing ionic strength of media containing acetylcholinesterase Changeux[115] has shown that an increase of ionic strength will increase the velocity of reaction but decrease affinity of substrates and competitive inhibitors. Through a

decrease in ionic strength he further demonstrated that flaxedil and d-tubocurarine are inhibitors of acetylcholinesterase. Irreversible acetylcholinesterase inhibition specifically at the anionic site of the enzyme was demonstrated for p-(trimethylammoniom) benzenediazonium fluoborate, and this enzyme inactivation could be prevented with the reversible inhibitor phenyltrimethyl ammonium chloride.[116] A number of other compounds have been shown to inhibit cholinesterases as dimethylsulfoxide,[117] and some isoquinolines.[118] Phosphorylated oximes inhibit acetylcholinesterase by mechanisms which are not different from the organophosphates.[119]

Acetylcholinesterase has also been shown to be increased by a de novo induced synthesis by actinomycin D. This synthesis of acetylcholinesterase is inhibited by puromycin and 5-fluororotic acid.[120] Acetylcholinesterase activity can be accelerated with acetyl fluoride in the presence of acetylcholine.[121]

Pharmacologic effects and interactions of esterase inhibition - The carbamate and organophosphate cholinesterase inhibition has been used extensively as a tool to study effects of changed or altered levels of esterase on animal behavior,[122] in nerve conductance,[123, 124] on body weight,[125] and on other systems briefly discussed below. Interactions between drugs are of current interest, as well as the effects of pesticides on drug actions and drug metabolism. The effects of pesticides (not the cholinesterase inhibitor type) on drug and steroid metabolism have been recently reviewed.[126] Since this review, it has been shown that the chlorinated insecticide, aldrin, could protect animals against the acetylcholinesterase inhibitors, such as parathion and physostigmine, for four days.[127] Furthermore, it was shown that there was first an increase in toxicity to chlorinated hydrocarbons followed by a decrease in the toxicity; this parallels an increase in hexobarbital sleeping time and then a decrease.[128] In this latter study it was shown that there was an increase of the carboxylic esterase "A" of the liver. With respect to this methods for separating the liver carboxylic esterases with organophosphates have been developed.[129] The chlorinated hydrocarbons also increase acute toxicity to several carbamates.[130]

The toxicity of the organophosphates by different routes of administration indicates that the liver can serve to increase toxicity, to detoxify and to serve as a buffering organ by absorbing certain carbamates and organophosphates.[131-133] A number of new toxicological effects of acetylcholinesterase inhibitors have been reported. The organophosphate, o-ethyl-o-p-nitrophenyl phenylphosphonothioate (EPN) has been shown to cause malformation in the developing duck foot due to a dystrophic effect on muscle.[134] Malathion will inhibit chick cell growth in tissue culture but will stimulate mouse liver cell growth in subacute toxic doses.[135, 136] Inhibition of erythrocyte cholinesterase from low doses of carbamates has been investigated,[137] and intensity of effects of carbamates has been compared to various mammalian esterases.[138]

Resistance to certain parasympathomimetics, such as carbachol, has been shown to develop with prolonged treatment with acetylcholinesterase

inhibitors in the cardiovascular system, isolated ileum,[139] and in the eye.[140] A number of miscellaneous effects and interactions with acetylcholinesterase inhibitors deserve mention. Central nervous system depressants cause an increase in toxicity to parathion.[141, 142] Physostigmine was found to directly decrease excitatory response to cholinergic stimulation and to cholinesterase.[143] The reaction rates for organophosphate inhibition of chymotrypsin and trypsin were determined.[144] Scopolamine-induced delirium was found to be antagonized by physostigmine.[145]

Choline acetylase - Much of the work of inhibitors for choline acetylase has been done before the period of 1966 with hemicholinium-like compounds. The mechanism of the hemicholinium-like compounds is thought to be either a block of the active concentrating mechanism for choline, because its action is so readily antagonized by choline, or it may compete with binding and storage of acetylcholine. Further studies of hemicholinium analogues have shown a wide variation of activity.[146] Hemicholinium-3 itself was found to have atropine-like activity,[147] and with vagus nerve blocking activity no reduction of atrial acetylcholine is seen,[148] nor is any change seen in acetylcholine containing vesicles in the caudate nucleus although there was a decrease in the parietal cortex.[149] The pharmacology of hemicholiniums has been recently reviewed.[150]

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Chapter 25. Current Status of Neurotransmitters

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Introduction - Although 46 years have elapsed since the relatively simple experiments of Otto Loewi demonstrated neurohumoral transmission at autonomic junctions[1], it is only during the past 10 years that this theory of neurotransmission has become almost universally accepted. Indeed, more evidence favoring the extension of this theory to nerve-nerve junctions (synapses) in the central nervous system (CNS) has been presented in the past 7 years than in the entire preceding quarter-century. Briefly stated, the theory of neurohumoral transmission holds that nerves produce their physiologic effects by releasing chemical substances which act on the cell that the nerve endings innervate, rather than by the continuous flow of bio-electric currents[2].

The identification of a putative transmitter substance as the neurotransmitter for a defined neural pathway (i.e., from cell type A to cell type B) is generally attempted by gathering data to satisfy criteria[3,4] in the manner of "Koch's Postulates" for the identification of a specific disease-producing microbe: 1) the putative transmitter must be detectable, together with the synthesizing and degradative enzyme systems, in the appropriate regions of the brain, or more precisely in the appropriate nerve endings. 2) The physiologic effect of the putative transmitter on the post-synaptic neuron must be identical to the effect produced by stimulation of the nerve pathway under investigation; depending on the electro-physiological finesse of the investigator, the effect of the putative transmitter may be compared on such general parameters as spontaneous or induced activity, or more intricately, on transmembrane permeability changes induced by the putative and true transmitters[4]. 3) Those drugs which either enhance or interfere with the response of the innervated neuron to the true transmitter must also influence (i.e., block or potentiate) the effects of the putative transmitter in an identical fashion. 4) When the pathway in question is stimulated, the substances released by activation of the nerve endings must be demonstrated to be identical to the putative transmitter; although constituting perhaps the most direct single piece of evidence, this criterion is the most difficult to satisfy in the CNS.

While a large number of substances have been proposed as neurotransmitters[5], only norepinephrine (NE) and acetylcholine (ACh) have been shown to satisfy rigorous criteria for a transmitter in the peripheral nervous system[2]. In the CNS only ACh[4,6] and possibly NE[7,8] and gamma-aminobutyric acid[9] can presently be accepted as likely transmitters for particular synaptic junctions, and the evidence for this comes mainly from extensive electrophysiological data.

Neuromodulation - It may be pertinent to point out here that some substances may act as neuromodulators in addition to or instead of acting as

neurotransmitters. The concept of neuromodulation, although not well defined, might be considered in the context of the modulator altering in some way the receptivity or reactivity of the post-synaptic element to the transmitter. Serotonin, for example, seems to have a buffer or modulator function in some peripheral systems with the possible exception of the clam heart, where transmitter function is suggested[11]. In the peristaltic reflex of the guinea pig, 5-HT facilitates the response of mechano-receptor sensory endings in the mucosa to changes in intralumenal pressure, inhibits the mediating "ganglion" (in Auerbach's plexus) and may facilitate response of serosal muscle to the transmitter (probably ACh) at the neuro-effector junction[12,13]. Similar effects of 5-HT in modifying the threshold for stimulation of cardiopulmonary and carotid sinus receptors have been demonstrated[14,15]. Evidence for a modulator action has also been obtained for histamine and NE at adrenergic ganglia and for NE at neuromuscular junctions (for discussion see reference 5).

Thus, in addition to the rather strictly defined concept of "neurotransmitter" given above, the conceptual possibility also exists that nerve stimulation to a tract of axons which densely innervate certain regional brain nuclei, might liberate substances that can act as local hormones by modulating activity, but without the requirement that these local hormones be the exclusive transmitter for any particular set of junctions.

Steps in Neurotransmission - At the outset it is important to detail the various steps in the synthesis, storage, release and destruction of neurotransmitters and their sites of action (receptors) on which this review will dwell. Very briefly these steps are thought to occur in the following chronological sequence:

1. Facilitated or active uptake of the transmitter precursor (tyrosine for NE and tryptophan for 5-HT) by the neural elements. In the case of ACh the uptake of precursors (choline and acetate) and the extent of recycling of these substances after hydrolytic cleavage of ACh are not yet clearly defined.

2. Biosynthesis of the neurotransmitter by enzymes which are either "soluble" in the cytoplasm or "bound" to certain subcellular organelles.

3. Uptake of the newly synthesized neurotransmitter by membrane-bound vesicles of various sizes and electron opacity, and storage of the neurotransmitter in a form bound to as yet undefined substances within the matrix of the storage vesicles. Other sites of storage also exist from which the transmitter may be more readily mobilizable. All of these various pools of transmitter maintain a dynamic equilibrium with each other.

4. Release of quanta of the transmitter by unknown physiological processes and association of the transmitter with the receptor macromolecule on the post-synaptic cell membrane. This

transmitter-receptor complex initiates a chain of events leading to depolarization or hyperpolarization of the post-synaptic cell, which in turn leads to its excitation or inhibition.

5. Very nearly simultaneously with the events in (4) occur the dissociation of the transmitter from the receptor and termination of its effect by enzymatic degradation or re-uptake by the pre-synaptic nerve ending or both. Some investigators[16,17] believe that the receptor and degradative enzyme are one and the same macromolecule in the case of ACh, so that receptor-response and hydrolysis do indeed occur simultaneously.

6. Repolarization of the post-synaptic membrane to a state of readiness for the next volley of transmitter molecules.

New Analytical Techniques - As with all explosions of interest and data in scientific areas, the special reason for recent successful experimentation in chemical synaptology is the discovery and application of a number of new and pertinent techniques. This review will be concerned with the new data which has emerged from the application of the following techniques to the problem of neurotransmitter identification in the CNS:

1. A variety of morphological techniques for directly localizing putative transmitters, such as:

   a) Histochemical fluorescence microscopy[18] and microspectrofluorometry[19] in the elucidation of monoaminergic fibers;

   b) electron microscopic analysis of nerve ending organelles in relation to storage and release of putative neurotransmitters[20];

   c) electron microscopic radioautography in the identification of specific monoaminergic fibers and storage of monoamines in the CNS[21,22,23];

   d) the localization of enzyme activities relevant to particular putative transmitters[24].

2. Micro-techniques for assaying the electrophysiologic effects of putative transmitters on single units in the CNS with respect to receptor activation and specificity of drug effects at possible receptors, especially the technique of microiontophoresis[25,26,27].

3. A variety of attempts to elucidate the release of transmitter substances after nerve stimulation[3,5,28,29].

4. The use of advanced neurochemical approaches on a multi-cellular level to identify and correlate various functional pools of transmitter substances, particularly the monoamines, with the effects of drugs and functional-behavioral manipulations, by

means of both isotopic and non-isotopic methods for examining turnover rates[30,31]

Each of these major areas of work relevant to the topic of neurotransmitters will now be considered, with the presentation restricted to the three monoamines which are currently prime candidates for transmitter roles in the CNS of mammals: ACh, NE, and 5-HT.

Cellular and Subcellular Localization of Neurotransmitters - When ACh was shown to be a transmitter at various sites of the parasympathetic nervous system, it was natural to analyze the brain for the presence of this substance as a preliminary qualification for its consideration as a central transmitter[32]. However, analysis of whole brain homogenates by bioassay[33], or even regional analyses of discrete CNS areas[34] by more refined extraction and identification techniques[35,36] can only serve to show differences in chemical content, and the crucial question remained as to whether the ACh in the brain was in nerves, and if so, in which nerves. It may be noted that the placenta is rich in ACh, yet no inference as to neurotransmission is associated with this chemical observation. In like manner, the same questions may be leveled at the chemical assays of NE and 5-HT in the brain, and it is in precisely this problem area that the morphological attempts at localization have been most heavily utilized. For all three amines considered in this review, the technique of differential centrifugal analysis of brain homogenates[37] showed that the monoamines were all found in highest concentration in that portion of the homogenate richest in particles which resembled sheared-off nerve-endings[38]; but obviously from an homogenate it is only rarely possible to identify the pathway from which the nerve endings were torn[39]. Therefore, the problem requires a technique capable of providing both chemical and structural information simultaneously.

Histochemical Localization of Neurotransmitters: For the catecholamines and indoleamines, much data are now at hand to establish not only the neuronal origin of the amines within the CNS, but also to describe the neuronal pathways involved[40,41]. This progress has been possible because of the development of a fluorescence histochemical method for these substances[42] and the method has been widely applied to evaluate the relative influence of an extensive assortment of psychopharmacological substances on the nerve endings and neuron cell bodies of the monoamine-containing cells[18,43]. Beyond this, however, the technique has certain limitations, particularly when the data are interpreted to yield statements regarding release and binding of monoamines with respect to synaptic function. Among the shortcomings of the method which have previously been cited[44,46] are the quantitative and qualitative estimation of the fluorophor in the tissue and the difficulty of identifying 5-HT fluorescent cell bodies in the absence of drug treatment. As the available technique is commonly applied, the observer's eye is used to estimate both the color of the fluorophor and its relative intensity, and it should be noted that the human eye is not an objective or accurate instrument for either measurement. The joint problems of quantitation and qualitative discrimination are complicated by the fact that large amounts of

fluorescence related to catecholamines sometimes give the apparent color of 5-HT (as in the adrenal medulla), and to the fact that both NE, dopamine and epinephrine have similar fluorescent emission peaks[47]. These problems have been circumvented in studies of the peripheral adrenergic system[48-50] by the use of microspectrophotometric measurements for obtaining exact activation and emission spectra of fluorophors, but a great deal of data, particularly pertaining to the CNS, prior to this latter technical sophistication, remains to be confirmed. Finally, it should be noted that the "system" of monoamine-containing neurons identified by fluorescence histochemistry are not always similarly recognized by neuroanatomists[51,52] and that the cellular morphology of freeze-dried tissues subjected to the fluorescence reaction leave much to be desired, particularly with regard to intracellular organelles.

Fine Structural Localization of Monoamine-containing Nerves: Fine structural analysis has the immediate advantage over the fluorescence approach in that it provides clearly superior resolution of cellular organelles, but at the same time requires multiple simultaneous maneuvers in order to provide chemical information on the cellular details visualized[53]. In the peripheral nervous system, fine structural analyses with a variety of fixation techniques and drug treatments have established that NE-containing nerve fibers exhibit synaptic vesicles with electron-opaque contents (dense-core or granular vesicles) and that the amount of granular material within the 400A° vesicles varies in good correlation with the NE content by both biochemical and fluorescent histochemical measurements[48,49]. However, from the available data, it appears that the material which becomes electron-opaque could be either the monoamine or the binding matrix for the amine[54]. In the central nervous system attempts to identify monoamine-containing nerves have also made extensive use of pharmacological depleting agents, particularly reserpine[55,56], α-methyl-m-tyrosine[57] and p-Cl-phenylalanine[20] as well as monoamine oxidase inhibitors[20,58] in efforts to observe changes in the frequency with which brain granular vesicles occur. However, although intriguing correlations can be shown between the regional concentration of NE and 5-HT and the frequency of nerves containing granular vesicles, most quantitative efforts to examine the problem fail to show any relation between the amount of granular material within the vesicles and the content of the amine after a variety of pharmacological manipulations in the amine levels[20,55,56]. The main problem has been the fact that in the CNS, the only form of granular vesicle found with routine types of tissue fixation has been a granular vesicle which is approximately twice as large as that studied in the peripheral nervous system[20,59], implying that fixation may be a critical factor in the preservation of the dense material which could represent the storage form of monoamine[20,60,61].

A second approach to the identification of monoamine nerve fibers in the CNS has been the use of electron microscopic autoradiography to identify those nerves capable of taking up and storing either H^3NE or H^3-5-HT after their injection directly into the cerebral ventricles[21,22]. The common result of all such studies thus far has been that the nerves which exhibit superimposed autoradiographic grains also contain the large

granular vesicles[23]. It has been suggested that the granular matrix of these vesicles might be a protein matrix used in the binding of the monoamine, but which does not vary in amount with fluctuations in the tissue amine level[20].

Enzyme Activity Localizations: An additional histological approach would be the ability to identify the intracellular location of enzyme activities related to one or another of the amines with which we are concerned. This approach has been used mainly for the acetylcholine-containing nerves[24,62,63] since there is at present no histochemical test for ACh itself. The histochemical demonstration of possible ACh-containing paths has rested upon the demonstration of cholinesterases by various light microscopic[62] and electron microscopic[24,63] techniques. Several types of brain cholinesterases with distinctive sensitivities to cholinesterase inhibitors[64] have been described by chemical characterizations, but it is not always possible to make these discriminations in the cytological tests. Furthermore, inasmuch as a variety of cell types unrelated to nerve function (such as pancreatic acinar cells and erythrocytes) also contain cholinesterase activity it is by no means certain that the presence of the enzyme is a valid indicator of ACh. By means of refined micromethods for the measurement of the ACh-synthesizing enzyme[65], it has recently been possible to show that the levels of the two enzymes are also not correlated with each other in a variety of sites in mammalian CNS[66]. Therefore, while it seems likely that the cholinesterase enzyme activity can be localized, it is not clear how significant these localizations may be to the determination of ACh-containing neurons[24,63].

Of the enzymes related to the catecholamines, methods have been presented only for monoamine oxidase[67] and this work has not yet been extended to the fine structural analysis of enzyme sites within nervous tissue.

Thus to summarize the present status of information derived from the histochemical experiments, we may conclude that neuronal circuits rich in acetylcholinesterase innervate the caudate nucleus[24], deep cerebellar nuclei[68], hippocampus[63] and possibly cerebral cortex[69]. Neurons containing NE and 5-HT arise mainly from the mid-brain and brain stem nuclei and course widely throughout the entire CNS[18,40], their synapses being particularly rich in basal ganglia[41], hypothalamus[41,70] and cerebellar cortex[71]. The key fact here is that these neurons contain the potential transmitter substance, and we shall now consider data concerning the response of the neurons in these areas to the substances.

Microiontophoresis - The characterization of a transmitter function for a chemical substance also depends upon the demonstration that its physiologic effects and sensitivity to drugs are identical to the natural transmitter. This statement implies that the putative transmitter substance is able to elicit some sort of physiologic response on the cell in question. In order to know this with certainty requires optimal physical resolution of the drug-neuron interaction and discreteness in both the route of administration of the substances tested and the delineation of

the neuron's responses[26]. This requirement arises partly because of the complex organization of nerve-nerve junctions, the presence of permeability barriers to the access of substances from the blood into the brain, and through the extracellular spaces of the brain to the active site, and also because neuronal events occur in matters of milliseconds, while substances injected into nervous tissue by parenteral or topical routes will require finite diffusion times which make it possible for observed effects to have been initiated many neurons away from the site of observation. In order to circumvent the major sources of diffusional barriers and to reduce the number of potential sites for drug-neuron interaction, the technique of microiontophoresis[25] was applied to the CNS as a method for administering small amounts of chemical substances into the immediate extracellular environment of neurons whose electrical activity could be recorded simultaneously and discretely[72]. As generally applied, 4 or more drug barrels containing ionizable substances are fused to a recording micropipette in such a way that the tips of all the pipettes are within 3-5 micra of each other[73]. When desired, the drugs in the side barrels are then ejected from the pipettes by passage of electrophoretic currents[74,75], although direct application of positive pressure[76,77] or electro-osmosis can be used for non-ionizable substances[78].

As with all methods, this too has its technical limitations[72]. Controls to rule out electrical current effects must be performed and the drugs must be prevented from leaking inadvertently from the pipettes when their administration is not desirable. In addition, although the amount released from the pipettes is small, the drug concentration in a small region may become relatively high. Since the extent of diffusion from the pipette is virtually immeasurable[79], the drug concentration at the site of action is also an unknown. Finally, since it is not possible to know with absolute certainty the anatomical location of the receptor site on the neuron in question (and in some cases not even the type of neuron under investigation), the interpretation of the direction of the response (i.e., activation or depression) may have to include the possibility of effects being mediated by an action on a near-by interconnected cell [72,80]. Effects which indicate artifactitiously positive responses may also be observed when a neuron is found to be responsive to a substance with which it is physiologically unlikely ever to contact[81,82].

When the data accumulated to date are considered from a statistical viewpoint, it appears likely that neurons sensitive to either ACh, NE, or 5-HT can be found in all regions of the CNS which have been analyzed[72,83]. The proportion of cells in any given region varies considerably, but when the neurons in a region are selected on the basis of their identifying response to a distinct pathway stimulus, the number of cells which are sensitive is higher[72,84]. Again from a statistical viewpoint, cells responsive to ACh can be either excited or depressed by it[72] while cells responding to either NE or 5-HT are usually[85] but not exclusively[86] depressed. When the available data are analyzed for completeness of electrophysiological responsiveness and complementariness of responses to agonist and antagonist drugs, consensual acceptance of ACh as the excitatory transmitter from motoneurone axon collaterals[4,6] and of NE as an

inhibitory transmitter from lateral olfactory tract axon collateral to mitral cells[7,8] represent the most satisfactory synaptic analyses. Less complete evidence would suggest ACh-mediated excitatory paths into caudate nucleus[87,88], onto cortical[72,89] and hippocampal pyramidal cells[72], for NE-mediated depression of spinal motorneurons[90] and for 5-HT mediated depression of lateral geniculate neurons[91,92]. In none of these cases are the histochemical and electrophysiological data complete but such combined approaches would seem to be essential, particularly in view of the difficulty encountered in attempting to demonstrate neuronally-mediated release of transmitters.

Demonstration of Release of Neurotransmitters - In the peripheral nervous system it was possible to isolate the innervated end-organ and to collect the vascular perfusate during nerve stimulation to associate the release of substances contained in the nerves with junctional transmission[2,3]. However, perfusion of specific local brain areas through their blood supply, with collection of the venous effluent for assay of liberated substances, is clearly impossible; furthermore, CNS stimulations are difficult (but not impossible) to conduct in such a way that the details of the neuronal pathway involved are fully known. Early approaches to this problem involved embedding minute polyethylene cylinders in specific regions of the cortex and collecting released material in saline irrigating the tissue under the cylinder[93,94,95]. It is of interest that by this approach it was possible not only to demonstrate spontaneous and evoked release of ACh from the cortex, but also to show that atropine and scopolamine, both powerful cholinolytic agents, also elicited a marked output of ACh by the cortex. This drug-induced release of ACh was direct confirmation of earlier findings[96,97] that a number of centrally acting cholinoceptive receptor blocking drugs would reduce the level of ACh specifically in the cerebral hemispheres. A recent analysis of findings by this technique[98] has demonstrated that in animals transected either at a midpontine pre-trigeminal level or at a collicular level, the activated electro-encephalographic (EEG) pattern was associated with a high release of ACh from the cortex, while a synchronized EEG pattern was associated with a reduced ACh release. These investigators also showed that scopolamine administered intravenously, intra-arterially or into the collecting cylinders caused a long-lasting increase in the spontaneous output of ACh. The most plausible explanation of this apparent release of cortical ACh by cholinolytic agents is that these drugs produce a net increase in output through occupation of specific ACh receptors. However, atropine in very high concentration failed to influence uptake of ACh by isolated slices of rat cerebral cortex[99]. Similar results with scopolamine on the uptake of ACh by nerve ending particles prepared from rat brain homogenates have recently been observed[100].

It was with the independent development of the "push-pull cannula"[101] and the chemitrode[102] that a technique was made available to collect and examine the local perfusate from brain nuclear masses. This technique, however, has been utilized surprisingly little. It was found that ACh was released when the tip of the cannula was in the cortex, but not when it was in the underlying white matter[103]. Furthermore, the rate of ACh

release was increased by stimulation of the cortex, injection of the convulsant drug, pentylenetetrazole, and stimulation of sensory nerves in the contralateral fore-paw. When the cannula was placed in the center of the caudate nucleus, the resting rate of ACh release was high (20 to 80 mμg/10 min), which was increased 3 to 8 times by stimulation of the anterior border of the primary motor cortex. By a similar approach, which consisted of 2 cannulas and 2 recording-stimulating electrodes, ACh (3 to 30 mμg/hr) was detectable on perfusion of the caudate nucleus, putamen, and subcortical areas of intact unanesthetized monkeys[104]. Seizures evoked by local electrical stimulation increased the release of ACh up to 375 mμg/hr[105].

More recent applications of cannulas of the push-pull type have indicated release from the brain parenchyma of either endogenous or radioactive amines after electrical stimulation[106,107] or drug treatments[108]. It should be borne in mind, however, that these cannulas, particularly when chronically implanted, lead to considerable tissue damage, casting some doubt on the significance of such types of "release".

Nevertheless, even the few data on the local release of ACh in brain induced either physiologically or pharmacologically, when added to the wealth of other data on ACh in brain, indicate a high probability that ACh does function as a transmitter at central synapses[109], and that pharmacological agents, which are active at peripheral cholinergic sites are able to exert effects by the same mechanisms in the CNS. A similar statement cannot be made with same degree of assurance for any other substance considered to be a central transmitter, although strongly presumptive evidence has been presented for 5-HT and NE in central neuronal systems[110,111,112].

By imposing even less stringent demands on the physiological nature of the neuronally mediated release, both NE and 5-HT have been shown to be releasable from isolated spinal cords[113] or from brain slices by electrical stimulation[114]. Release has also been studied by indirect methods in vivo by analyzing amine levels and related substrates after more or less physiologic electrical stimulation of the cell body regions[115,116,117]. Here release is inferred by modification of the fluorescence histochemical picture or by changes in the amounts of transmitter metabolites, but such changes may well occur exclusively within the nerve ending and do not require that the nerve ending actually released the substance. Clearly a cellular approach to the question of release is essential.

Multi-cellular Biochemical Synaptology - While it is clear from the previous material that the critical assessment of neurotransmitter functions for ACh, NE, or 5-HT is almost completely lacking, it is also clear that these deficiencies are technical. On the other hand a variety of drugs such as reserpine[118], amphetamine[119], imipramine[120], chlorpromazine[110] and various psychotomimetics[121,122] appear to be involved with the neuronal metabolism of NE and 5-HT. It may be tentatively inferred, then, that biochemical studies of the metabolism of these substances, when interpreted in the light of the previous data, may help to provide insight into

the subcellular mechanisms of action of these drugs. That is, if the behavioral effects of reserpine are mediated through neurons whose synapses transmit by the release of either NE or 5-HT, the biochemical results of reserpine treatment might be discernible from the examination of the turnover rate of the various functional metabolic pools of these monoamines[112,123].

Very early considerations of the subcellular status of brain monoamines were concerned with two postulated pools: one representing the storage compartment of the amine bound in an inactive form to some cellular components and the other representing a freely diffusible, active form of the amine[124,125,126]. In addition to the problems concerning the identification of the one or more intra-cellular storage particles is the clarification of the number and inter-relationship of the chemical "pools" of stored amines. Based on the centrifugal analyses defining "soluble" and "particle-bound" amine, the concept of vesicular and extra-vesicular amine pools has been repeatedly proposed[127,128], but with considerable lack of agreement on the exact proportion represented in each compartment. Moreover, beyond the concept of vesicular and extra-vesicular pools, there is also partial evidence in favor of the existence of pools representing storage of recently synthesized amine, recently incorporated amine and more or less tightly bound amine (cf. 29). Despite the fact that these various sub-compartments are identifiable in functional and not morphologic terms, the probability of their existence makes it no longer possible to attach significance to alterations in total levels of the amines as correlates of altered function. Therefore, the effect of many centrally acting drugs on total levels of brain amines must now be re-examined in the light of dynamic aspects of monoamine metabolism in the brain, since the storage levels of these amines are not static but reflect dynamic equilibria between their rates of formation and their rates of utilization[85,129].

The turnover of monoamines has been estimated by measuring the rate of decline of specific activity of the amines after introducing small amounts of radio-isotopic amines into the endogenous pools. This technique was first used to measure the rate of turnover of adrenal medullary catecholamines after the administration of radioactive precursors[130]. The problem of the relative impenetrability of the blood-brain barrier to peripherally circulating amine was circumvented by the development of techniques for the administration of radioactive NE into the lateral cerebral ventricle[131], which allows a direct labelling of brain catecholamines. The principle of this approach is to introduce a "pulse dose" of labelled NE into the endogenous stores of this amine in the brain, and to follow the rate at which the radioactive material is catabolized and becomes diluted with newly synthesized NE[129,132]. Similar studies with H^3-5-HT have also been reported[111,133].

Other approaches to this problem of amine turnover as applied to catecholamines in brain have been to follow either (a) the rate of disappearance of endogenous NE after inhibition of catecholamine biosynthesis by α-methyl-p-tyrosine[134] or (b) the rate of disappearance of synthesized

radioactive NE after the intraventricular injection of ^{3}H-dopamine[30,129]. Regardless of the technique used, there were no significant differences in the estimates of NE turnover[129]. ^{3}H-NE demonstrated a multi-phasic disappearance from whole brain, and this is taken to indicate that the labelled amine is retained in more than one pool. On the other hand, more than 90% of the ^{3}H-NE accumulated in brain seems to be retained in a single compartment from which it disappears with a single exponential decay rate during the first 8 - 12 hrs. after administration. The slope of this major decay curve indicated a half-life for NE in brain of about 4 hours. Tritiated-5-HT injected into the lateral ventricles of rats is also taken up by the brain and declines with a half-life of 4 to 5 hr.[133].

Another technique[36] for measuring turnover time of 5-HT makes use of a blockade of monoamine oxidase with a number of inhibitors (including pargyline and tranylcypromine) and calculation of the rate of synthesis from the product of the rate constant of 5-hydroxyindole acetic acid (5-HIAA) decline and the normal 5-HIAA level. On the other hand, because of variation seen in the rate of increase in NE after inhibition of MAO in different species, it has been inferred that the rate of increase in NE after blockade of MAO is not a good measure of rate of synthesis of this amine[37].

With the establishment of these techniques for studying amine turnover, a variety of procedures and drugs on such turnover has been investigated. There was an increased rate of ^{3}H-NE in the brains of rats subjected to intermittent electric shock to their feet[135]. This was interpreted as a sustained increase in the synthesis and utilization of NE in the brain. In addition, an increased turnover of NE was observed in the brains of rats during the rebound phase following REM-sleep deprivation[136]. After depletion of 5-HT stores by reserpine the rate of 5-HT synthesis is not decreased, but may even be increased[137,138,139].

Summary - It is clear that the elucidation of the mechanism of action of centrally active drugs, cannot yet be conclusively related to the synaptic actions of particular neurotransmitters. However, the continued application of constantly improving technology provides greater hope of a "breakthrough" than ever before in this area. In addition, the increasing sophistication of technique is making possible a clarification of the nature and role(s) of neurotransmitters in the CNS.

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Chapter 26. Drugs and Memory and Learning

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Introduction - Another general survey of this subject would be gratuitous. There is a plethora of recent, often exhaustive reviews of the various overlapping sub-areas that comprise biological approaches to learning and memory, i.e., drugs[1-3], electroshock and "consolidation" of memory[4-12], biochemical theories[8,13-20], neurophysiological theories[3,4,8,9,21-25] and effects of trauma and neuropathy[21,22,26-31]. A few up-to-date reviews are especially recommended for the uninitiated: John's volume[22] and Part 4 of Grossman's ambitious text[32] present excellent and complete accounts of contemporary theories--with ample source material--on biological bases of learning and memory. Efron's philosophical scrutiny[33] of recent biochemical theories and their experimental props adds a needed and readable critical perspective for any newcomer to the field.

The present discussion will concentrate on a single component of recent learning and memory research, one which perhaps most immediately concerns the medicinal chemist: the current status of drugs which have been purported--indeed, sometimes heralded--to facilitate memory and learning.

Current Status of Drugs Purported to Enhance Memory and Learning

Before proceeding to the drugs themselves, let us consider in the briefest and most general way their sources and rationales. Details can be found in references cited above.

This reviewer is unaware of any reputedly active memory and learning drug which was selected empirically, either in man or in laboratory animals. One might assume that if any common clinical agent in fact enjoyed such activity, intellectual enhancing effects would be uncovered by clinical accident. Comparable accidents, after all, led to the discovery of such centrally acting drugs as chlorpromazine, iproniazid and imipramine. Similarly, it is not altogether unfeasible that a behavior scientist in a drug environment might have chanced across a new compound that enhances acquisition in animals. Discovery of other drugs by this route is not uncommon.

But instead, experimental compounds of interest in memory and learning research have all derived from well-verbalized, but necessarily poorly founded, theories. Often the investigator sought to use a drug primarily to buttress a theory. And foremost among current theories are those concerned with "consolidation" mechanisms for the "memory trace". A wealth

of experimental data and clinical experience has been interpreted as showing that memory formation can be subdivided into at least two components. The first of these is a labile, short-term phase usually thought of as electrical. Certain treatments, most notably electroconvulsive shock, can disrupt memory in this phase, thereby producing "retrograde amnesia". The second phase, resistant to disruption by electroconvulsive shock, although other amnestic treatments are sometimes claimed to be disruptive[34,35], may be described as permanent, long term or "consolidated" memory. Most prominent among the various substrates proposed to account for such long term storage are intraneuronal mRNA molecules[13]. Facilitation of events at the still hypothetical interface between the assumed electrical substrates of short term memory storage and the assumed biochemical substrates of long term memory storage, in other words facilitation of the "consolidation" process, has been tacitly chosen as the favorite recent rationale for drug discovery. Most drugs currently advanced as enhancers of memory and learning thus either stimulate electrical processes in the CNS (e.g. strychnine) or enhance (at least in claims) RNA formation or concentration in brain (e.g. magnesium pemoline, tricyanoaminopropene, RNA itself).

Strychnine and Other Analeptics - McGaugh and Petrinovich[1] have already reviewed an extensive series of studies, performed mostly by themselves, their colleagues and their students, showing enhancement of acquisition in animals by strychnine. Reports alleging such an effect are not new. Lashley[36] in 1917 found strychnine to enhance maze learning in rats. But McGaugh's group has added several important experimental modifications. Perhaps the most important of these is the use of post-trial injections, that is, administration of drugs shortly after the animal is exposed to the learning situation. Such treatment is intended a) to avoid the interpretive complications of training animals under the drug and b) to stimulate neural processes during the so-called period of consolidation.

Positive findings on effects of CNS stimulant drugs on learning in animals have not been limited to strychnine. Comparable reports have also appeared of learning enhancement following post-trial treatment with such analeptics as diazadamantanol (1757 I.S.: a strychnine analogue)[37,38], picrotoxin[39-42], pentylenetetrazol[43], and even amphetamine[44] and caffeine[45].

McGaugh's speculations on the significance of learning enhancement by strychnine primarily concern its CNS stimulant action. A conceivable link between the effect of strychnine and enhanced synthesis of RNA can not, however, be ruled out. Strychnine has in fact been reported to elevate RNA content in rat brain[46,47] by unknown mechanisms.

Several studies[48-51] are inconsistent with the positive results of McGaugh and his coworkers. For example, Prien et al.[48] saw no enhancement effect from either intraperitoneal strychnine or picrotoxin in a careful rat maze study, and Stein and Kimble[49], also using a maze, failed to find facilitation with post-trial strychnine injections. McGaugh[6] and Petrinovich[52] attribute these findings to problems of dosage or rat strain,

and have admirably presented direct data in support of their contention. It may be assumed, however, that the usual negative study "iceberg effect" applies; for every negative finding which surfaces in a published report, a much larger number of unpublished negative results exists.

But the chief practical defect of the animal data on strychnine--as well as those on most other analeptics--is the dearth of relevant human data, or even of data in species other than rats and mice. Caffeine and amphetamine, however, have been subjected to many older human studies concerned with effects on learning. The most authoritative review of this literature[53] concludes that "the evidence at hand...indicates that neither amphetamine nor caffeine possesses properties which lead to improved intellectual performance except, perhaps, when normal performance has been degraded by fatigue or boredom."

"Naive" RNA - With the proliferation in the early 1960's of theories linking RNA to memory it was perhaps inevitable that RNA itself would be tested for its facilitative effects on learning. Present interpretations of such experiments, and there have been several in animals and humans, must be tempered by the recognition that exogenous RNA introduced by other than intracerebral routes does not enter the CNS intact[54-57]. Obviously, then, positive results can only be attributed to fragments of RNA which may enter the CNS, or to indirect sequelae to peripheral effects.

The most frequently cited study of effects of RNA on memory is that of Cameron and Solyom[58] in humans. These authors claimed that in aged patients yeast-derived RNA exerts a favorable effect on retention. Alertness and interest were also enhanced, and positive results were seen in a double blind, as well as in an uncontrolled study. Remarkably, RNA given orally was effective, though less so than intravenous RNA. Other studies by Cameron and his associates[59-61] also yielded mostly positive results. However, recent attempts to replicate the positive effects on learning and memory have been unsuccessful[62,63]. In the former of these studies[62], a suggestion is advanced that unusual attention to senescent patients in Cameron and Solyom's studies may have accounted for the favorable effects; this, however, ignores the double blind study in their initial report. In the second study[63], done by Cameron's former coworkers, no evidence of enhanced memory function was found, but the dosage did not reach the high levels (a total of 700 g. over a 3-month period) used by Cameron and Solyom[58].

The rat study of Cook *et al.*[64] also gained wide attention. These authors reported that prolonged administration of yeast-derived RNA increased the rate of learning in a pole-jump avoidance task; resistance to extinction was also increased in the RNA group. Two confirmations of these results have been reported[65,66], but in each of these studies the effect enjoyed very little generality. Other acquisition tasks, including shock avoidance procedures, were not enhanced by yeast RNA. Other negative reports[67-71] also suggest that if RNA is effective in animal learning studies, it is an effect highly specific to the procedure used by Cook *et al.* Of these studies a few are of special relevance:

Boissier et al.[67,69] went to some lengths to replicate the dosage protocol used by Cook et al.[64] but obtained only negative effects in a shuttle-box avoidance situation. Beaulieu[70] could not demonstrate a blockade by RNA of electroconvulsive shock-induced retrograde amnesia; in fact, an enhanced amnestic effect was seen. Two operant conditioning studies[72,73] indicate that RNA elevates response rates on a variable-interval reinforcement schedule in rats, curiously suggesting that a stimulant action resulting from peripheral administration of RNA might have to be reckoned with in interpretations of learning data.

"Trained" RNA and "Learning Transfer" Experiments - This highly controversial subject is considered to fall beyond the scope of the present review. One justification is that the polypeptide-, protein-, nucleotide- or RNA-rich brain homogenates (from trained animals) usually employed in vertebrate experiments can scarcely be considered drugs. Another is that few clarifications have appeared in this field since the excellent critical reviews by Booth[13] (see especially p. 162) and Grossman[32].

Magnesium Pemoline - Although pemoline (Cylert) had previously been marketed in Europe for some years as a stimulant and "antifatigue" agent, serious interest in its effects on learning and memory did not begin until early 1966. In consecutive articles in Science, Glasky and Simon[74] reported that magnesium pemoline stimulates brain RNA polymerase in vitro, and Plotnikoff[75] reported it to enhance acquisition and retention of a conditioned avoidance response in rats. In each case, the action of magnesium pemoline was not shared by other CNS stimulants. The inference that the two effects might be related was clear. The suggested memory-enhancement properties of magnesium pemoline, along with their theoretical implications, were accepted at face value and heralded in many scientific and lay publications. Plotnikoff has since further claimed a) that the compound "enhances consolidation of 'memory storage' processes"[76], since the effect on retention occurs when injections are given following acquisition trials (cf. McGaugh and Petrinovich[1]), b) that magnesium pemoline is more potent in enhancing retention than is pemoline itself, although pemoline is active[77], and c) that magnesium pemoline enhances retention and acquisition of a conditioned photic response in rabbits[78].

The biochemical findings of Glasky and Simon have not been confirmed by other groups. On the contrary, three laboratories have reported negative or inconsistent data. Cain[79] found that pemoline does not stimulate RNA polymerase in vitro as assayed by incorporation of [^{3}H] uridine into brain slices. Morris et al.[80] reported a similar negative result, including an in vivo study. And perhaps most discouraging, though admirable, was a detailed report[81] from the same laboratories from which Glasky's initial work derived. The conclusion: "repeated attempts...to reproduce the original experimental observations [of Glasky and Simon] have been unsuccessful".

Similarly, Plotnikoff's avoidance studies and his interpretations of them have not withstood attempts at replication and reanalysis. No fewer than ten recently published behavioral studies[82-91] have attributed

reductions in avoidance latencies in mice or rats to a stimulant action of the compound on performance, rather than to an enhancing effect on acquisition or retention. Other interpretive possibilities have also been proposed, for example, that magnesium pemoline acts in avoidance situations by reducing emotion-induced immobility ("freezing") of the animal[84,92], that the original effect on retention reported by Plotnikoff confuses a true retention effect with an artifact resulting from short acquisition latencies[93], and that pemoline-treated rats may be more sensitive to a conditioned stimulus or to foot-shock[92]. With respect to this last point, behavioral studies by Tenen[94] on the tryptophan hydroxylase blocker, p-chlorophenylalanine, are noteworthy. This drug was found to accelerate learning by rats of a shock-avoidance task similar to the one used by Plotnikoff, but careful behavioral analysis revealed that the effect is probably caused by a hyperalgesic action, making foot shock more aversive. This is precisely the type of analysis lacking in the initial behavioral studies on pemoline.

If magnesium pemoline has a generalized acquisition-enhancing effect in rats, independent of its stimulant effect, one might also expect it to facilitate acquisition under conditions where response restraint indicates appropriate behavior, as well as in "rapid response" situations. But only negative results have been reported[85,86,95].

Efforts to achieve an acquisition-enhancing effect with single doses of magnesium pemoline in humans have also encountered negative results. Talland[96], using student volunteers, found enhanced attention after magnesium pemoline on a task entailing rapid digit counting and vigilance, but decided that the drug effect was related to its stimulant action and not to an effect on memory processes. Three studies using widely varied learning tasks in normal humans found acutely-administered magnesium pemoline to exert no prominent learning enhancement effect[97-99].

Cameron's[100] criticism of one of these studies[99] applies to all: chronic dosage may be essential for an effect in man. Cameron[100] in fact presented data--although without protocols--asserting elevated IQ scores in elderly patients with memory defects after one month of continuous pemoline therapy. This reviewer believes that Cameron's assertion should be regarded very cautiously in the absence of verification. Using patients with Korsakoff's syndrome, including one whose syndrome was not a sequel to chronic alcoholism, Talland et al.[101] failed to see a useful effect of magnesium pemoline, even after 3 weeks of daily administration. Their conclusion, "the day that offers a pharmacological remedy for severe memory disorders has not dawned yet, and it is unlikely that Cylert will prove to be the much-hoped-for cure", offers an appropriate departure point from the current status of magnesium pemoline.

Tricyanoaminopropene (TCAP; U-9189) - Like magnesium pemoline, TCAP attained prominence as a potential learning-enhancing compound following a reported effect on neural RNA. It had initially been reported that the common intermediate, malononitrile, stimulates nucleic acid production in

the CNS[102], but chemical analyses[103,104] suggested that a dimer, TCAP, rather than malononitrile itself, was the active agent. Egyhazi and Hyden[105] indeed confirmed that TCAP in rabbits increases nerve cell RNA by 25% in Deiters cells; changes in RNA base makeup were also reported. No independent confirmations of these findings have appeared. Two studies, however, are consistent with the Egyhazi and Hyden data. Jacob and Sirlin[106] found pronounced elevations *in vitro* in RNA concentrations in the salivary glands of an insect, and Essman[107] reported elevated total RNA concentrations in several parts of mouse brains after TCAP treatment.

TCAP was first tested for effects on retention by Chamberlain *et al.*[108]. The compound was reported to enhance retention of an avoidance response in rats under conditions where possible "emotionality" or "arousal" effects could be discounted. In addition, TCAP was found to accelerate "fixation" of a postural asymmetry in an unusual rat preparation, an effect which could conceivably be considered enhanced "consolidation" of memory (cf. Weissman[2], p. 193).

Since the studies of Chamberlain *et al.*[108] appeared, several attempts to duplicate or extend the behavioral data have been made. Most of the published reports have yielded positive results. In "dull" or "average" rats TCAP has been reported to reduce errors in a visual discrimination T-maze[109], and in rats chronically treated with TCAP, facilitation of a spatial discrimination task and of reversal training was seen[110]. In this latter study, remarkably, acquisition was apparently enhanced despite the functional cretinism induced by the anti-thyroid effect of TCAP[111,112]. No such symptomatic manifestations were reported by Essman[113], who treated neonate mice up to 16 days of age with TCAP, and who noted improved acquisition of a water maze at 25 days, that is, 10 days after the last TCAP treatment. Interestingly, these 3 behavioral studies are inconsistent with the one negative result reported by Chamberlain *et al.*: that TCAP did *not* enhance acquisition in a maze. Additional negative findings[114] have since also been reported.

Essman[107,115] has offered evidence of a different type in favor of an acquisition enhancing effect of TCAP. Mice given TCAP and then exposed to very rapid learning (a "1-trial passive avoidance situation") appeared resistant to the retrograde amnesic effects of electroconvulsive shock; rat data in the same direction as these findings have also been reported[2]. Nevertheless, the data are probably not yet firm enough to warrant Essman's[107] conclusion "that an acceleration of RNA synthesis with TCAP serves to facilitate memory trace formation."

Davenport (cf.[110], footnote 2) has made available an unpublished summary (dated Aug. 14, 1965) of a substantial number of behavioral studies on TCAP, all conducted at the Wisconsin Regional Primate Research Center. Most of these used rats, but some used squirrel, stump-tail, or rhesus monkeys. Considerable variation in the effect of TCAP was seen, and in some studies even decremental effects on acquisition and retention were indicated. Nevertheless, facilitative effects were often seen, especially when dosage was chronic.

Two published studies of the effect of TCAP on acquisition in humans, both discouraging, have appeared. In one[116], 23 senile patients with memory deficits were exposed to a 2-week course of treatment. No evidence of improved acquisition or retention was seen in a variety of tasks at which these subjects were normally markedly defective. In the other, rather poorly described experiment[117], negative results were obtained from aged patients given a chronic course of treatment. As yet, then, the medical potential of TCAP must be considered doubtful.

Miscellaneous Compounds - Simon et al.[118], the same group which initiated biochemical interest in magnesium pemoline as an enhancer of RNA polymerase activity, have very recently announced the discovery of ribaminol, a salt of RNA and diethylaminoethanol. According to Simon et al., this salt, (unlike its two components) "stimulates in vivo and in vitro the systems in brain responsible for protein synthesis." The authors further assert that "ribaminol has been shown to enhance the acquisition of learned responses under a wide variety of conditions." Glasky has presented these learning experiments[119], which include a study in humans, and they are not without complications. For example, Glasky suggests that females (both rats and humans) are responsive, but males of each species are not; in fact, males exhibit a performance decrement, possibly as a result of excessive dosage. Furthermore, only certain tasks exhibit improvement under drug, and dosage and duration of treatment are critical. Ribaminol will inevitably enter the treadmill of diverse biochemical and behavioral replicative attempts, just as pemoline has done. Time will tell whether the eventual appraisal by disinterested investigators will be more positive than it has been for pemoline.

Deutsch and his colleagues[120-122], have reported that under restricted conditions (partially-trained subjects, injected on the right day after training) rats can recall a maze task more accurately if the cholinesterase blocker, DFP, is administered intrahippocampally. In well-trained rats, however, or in rats injected at a sub-optimal time, DFP exerts a deleterious (amnesic) effect on recall. These abstruse results are obviously of little practical value until further statements of their generality can be made.

The current, unencouraging status of certain older drugs which were at one time reputed to enhance acquisition--thiamine, glutamic acid, nicotine--has not changed significantly since the review by McGaugh and Petrinovich[1], which may be consulted for primary sources.

General Assay Considerations

The preceding sections make it clear that none of the compounds presently in the limelight as potential memory enhancers has given reasonable evidence of positive effects in man. In addition, the animal results are all too often controversial. It is of course true that those negative results which are available do not "prove" inactivity, but one must conclude that no reliable comparison standard is available. It follows that animal tests cannot now be validated by the use of known actives. In the

case of antipsychotic drugs, the availability of chlorpromazine enabled partial validation of, for example, conditioned avoidance and anti-amphetamine procedures; in the case of antidepressants, the availability of imipramine enabled partial validation of reserpine antagonism procedures and tests for blockade of norepinephrine uptake; in the case of antianxiety drugs, the availability of chlordiazepoxide enabled partial validation of anti-pentylenetetrazol and anti-"conditioned emotional response" effects. But no such drug tool buttresses memory and learning research.

In the absence of any possibility for "empirical validation", reasonable animal procedures must necessarily offer "face validity", that is, rationales which relate the assays to acquisition in man. And once this is agreed, investigators can not avoid consideration of the many pitfalls in the interpretation of animal learning experiments, the most common being a confusion between drug effects on performance and drug effects on learning[1]. Obviously, no animal tests can parallel certain common learning and memory phenomena in man. As Weiskrantz[4] points out, for example, "free recall"--perhaps the most common type of memory test in man--has no apparent animal parallel. But some animal tests do bear an analogy to human situations, for example, retrograde amnesia experiments[2]. Such models must probably comprise the core of any preclinical testing program.

Validity is not the only need of an adequate test; reliability is equally important. The behavior scientist's excuse for inability to reproduce results seems too often to be: Conditions were slightly different, doses differed slightly, the strain of animal is all-important, etc. Some burden would appear to rest on any discoverer of a drug-induced memory-enhancement effect a) to reproduce his results carefully, and b) to pursue as far as is practicable the generality of the effect, for example, at several doses, in a variety of species, and in several tasks.

A third requirement of sound assays is practicality. In memory and learning research training procedures are complex and, because of the variability inherent in this research, group sizes must be substantial. Furthermore, by the very nature of the problem, one test of a well-trained animal may exhaust his usefulness. Methodological improvements are needed to contract the time scale of behavioral testing to realistic durations. One-trial learning procedures, often used in retrograde amnesia experiments, can be useful. Delayed matching procedures, in which subjects can be repeatedly used, exemplify another type of practical procedure. When practical learning and memory tasks are introduced perhaps a modicum of largely empirical drug assay in animals can proceed. At the present time any truly active drug would make a substantial contribution to theory, regardless of potential utility in therapeutics.

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Section VI - Topics in Chemistry

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Chapter 27. Synthetic Approaches to Prostaglandins

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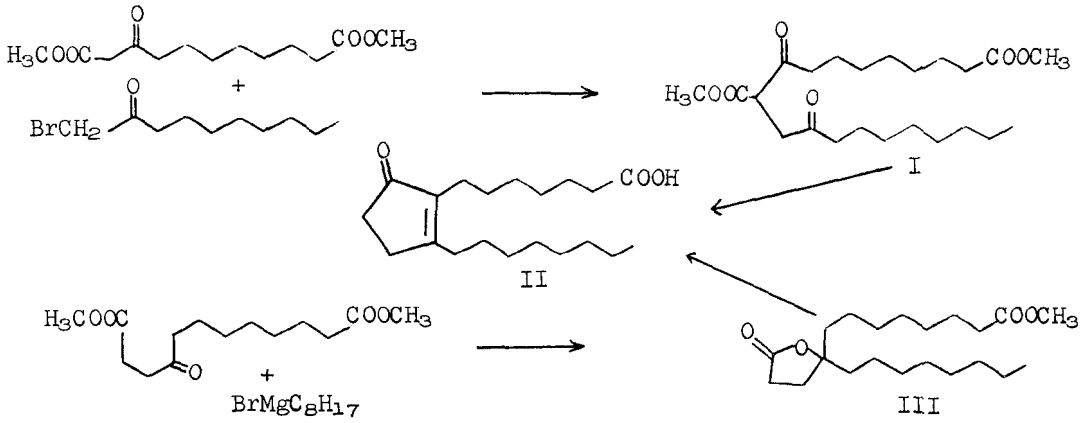
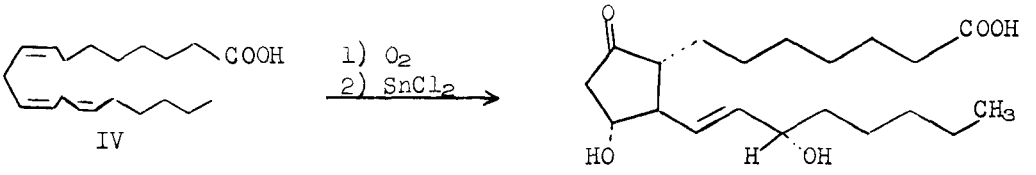
The interest in the field of prostaglandins is rapidly increasing as demonstrated by a growing number of publications (156 in 1967 compared to 105 in 1966 and 73 in 1965). Two books[1,2] published in 1967 cover to a large extent our present knowledge of the chemistry, biochemistry and biology of the prostaglandins. In addition, several articles[3,4,5,6,7] have appeared which provide an exhaustive review of this new class of compounds covering especially their biochemistry, biology and pharmacology. Together with the references cited therein, they provide a convenient introduction to this field for the interested reader. The major handicap in prostaglandin research is still the limited amount of material available. Natural occurrence although ubiquitous in mammalian tissues is ruled out as a practical source of large amounts because of the low concentrations in which they are present. Bioconversion of unsaturated fatty acids, the natural precursors of prostaglandins[8,9], yields prostaglandins in reasonable amounts[4], but is limited by high costs and the amount of seminal vesicles available. Therefore, total synthesis is considered to be the ultimate source for these natural products. The major breakthrough in this direction has yet to come but several groups are working intensively toward this goal.

The first synthesis of the prostaglandin skeleton was described by Samuelsson and Ställberg[10] in connection with the structure elucidation of prostaglandin E_1 (PGE_1) V. Alkylation of methyl 5-ketoundecane-1,11-dioate with 1-bromodecane-2-one yielded diketone I, which was converted to 15-desoxy-13,14-dihydroprostaglandin B_1 II via base catalyzed cyclization,



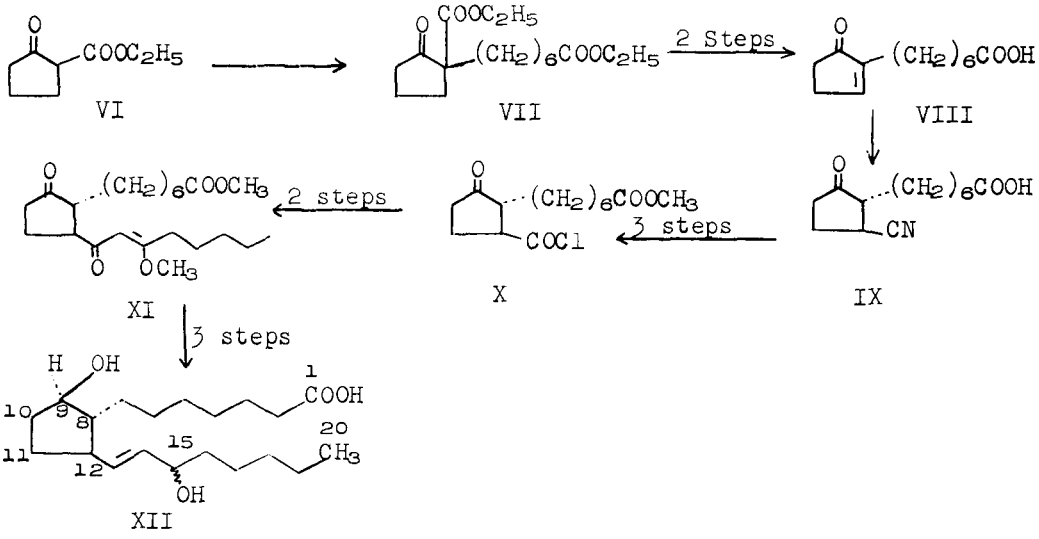
ester hydrolysis and decarboxylation. The same product was obtained by a Grignard reaction of 4-ketododecane-1,12-dioate with n-octyl magnesium bromide and cyclization of the resulting γ-lactone III. II proved to be identical with a degradation product of natural PGE_1.

Imitating the biosynthesis of prostaglandins the Unilever group[11] obtained prostaglandin-like material in 1-2% yield by autoxidation of all-cis-8,11,14-eicosatrienoic acid (dihomo-γ-linolenic acid) IV followed by reduction with $SnCl_2$. About 5-10% of this material was identified as d,l-PGE_1 V.



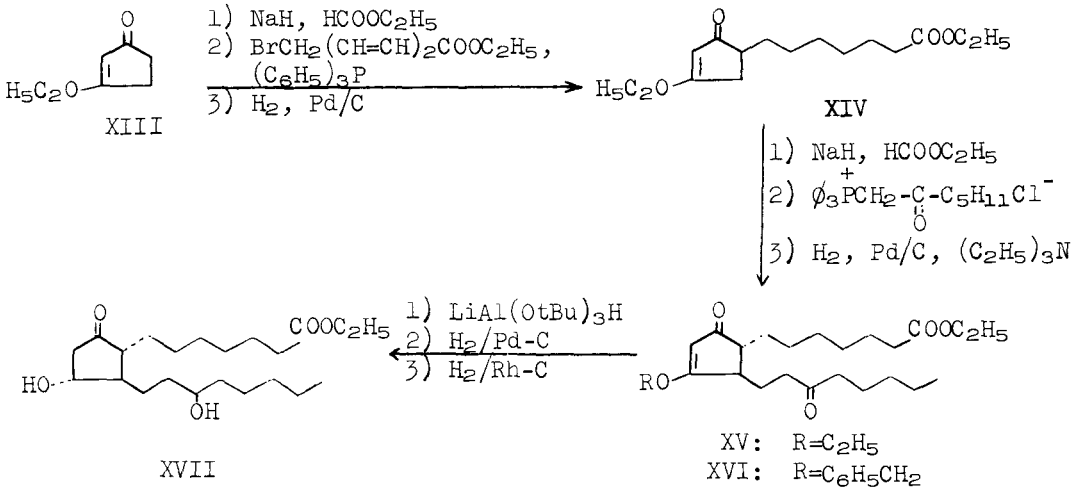
V: d,l-PGE_1

Bagli and coworkers[12,13] at Ayerst were the first to report the total synthesis of a pharmacologically active derivative of prostaglandins. Starting with ethyl 2-cyclopentanone carboxylate VI d,l-11-desoxy-prostaglandin $F_{1\beta}$ XII was prepared in a 12-step synthesis. Noteworthy is the



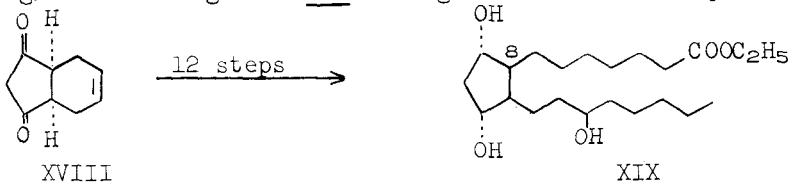
construction of the olefinic side chain: Bromination and acid treatment of intermediate VII gave cyclopentenone VIII which was converted to nitrile IX. IX was converted into acid chloride X, insertion of heptyne followed by base treatment yielded enol ether XI, ester hydrolysis, $NaBH_4$ reduction, dehydration and second $NaBH_4$ reduction gave the end product XII, a mixture of alcohols epimeric at C-15.

The first naturally occurring prostaglandin was synthesized by Beal, Babcock and Lincoln[14] of the Upjohn laboratories. Starting with 3-ethoxy-2-cyclopentenone XIII d,l-dihydro-prostaglandin E_1 ethyl ester XVII was obtained as one isomer of a complex mixture of isomeric racemates. Structure and stereochemistry of XVII was proved by a number of physical, chemical



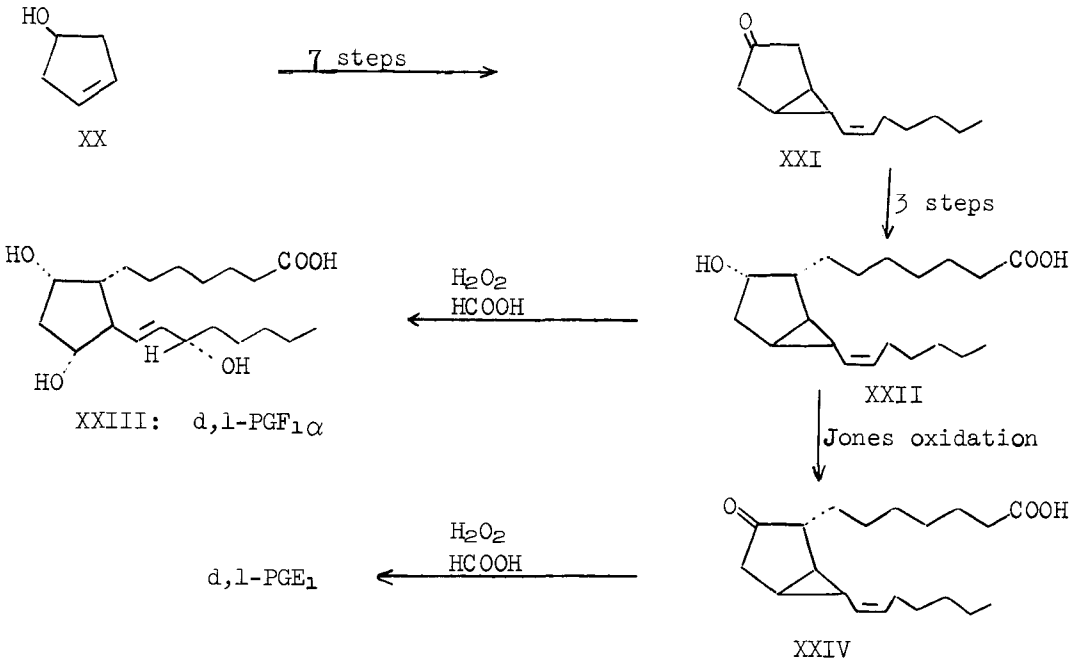
and biological methods, comparison with the ester of hydrogenated natural PGE_1, and unambiguously confirmed by radioisotope dilution. An interesting feature of this synthesis is the large number of highly selective reductions and hydrogenations. Each of the two side chains is introduced into the five membered ring via formylation followed by a modified Wittig reaction.

In another approach by the same group[15], a Diels-Alder reaction was utilized for the introduction of both side chains which led to a synthetic prostaglandin analog with cis configuration of the alkyl side chains. The



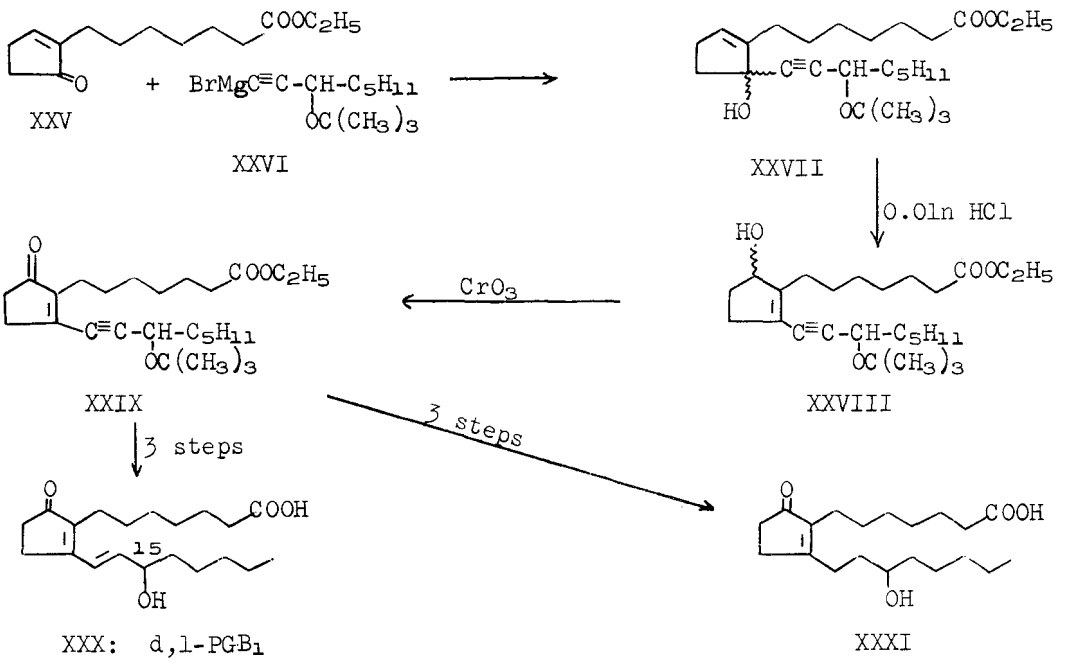
Diels-Alder adduct XVIII of cyclopentene-3,5-dione and butadiene was converted to the "8-cis" analog of dihydro-prostaglandin $F_{1\alpha}$ ethyl ester XIX in 12 steps.

A synthesis of d,l-prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) was described in a preliminary communication by Just and Simonovitch[16] at McGill University. Cyclopentenol XX was converted to the bicyclic ketone XXI in seven steps. Alkylation of XXI with methyl 7-iodoheptanoate followed by $NaBH_4$ reduction and ester hydrolysis yielded acid XXII. Oxidative formolysis,

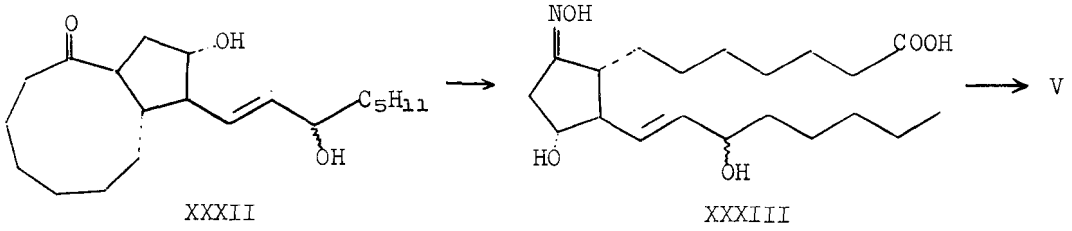


the key step of this synthesis, gave d,l-$PGF_{1\alpha}$ XXIII according to TLC, mass spectrum and preliminary biological data. TLC analysis of an impure product obtained by oxidative formolysis of XXIV indicated the presence of d,l-PGE_1 V. A very recent publication by a group at Smith Kline and French laboratories[17] describes a repetition of this approach and lists some differences from the McGill work particularly regarding the stereoselective nature of the synthesis and the ability of the last step to give either d,l-$PGF_{1\alpha}$ or d,l-PGE_1. A modification did, however, produce d,l-PGB_1 (XXX). Presumably a resolution of these discrepancies can be achieved and the potential of this overall scheme assessed as a workable synthesis when the full papers are published.

Hardegger, Schenk and Broger[18] published a straightforward synthesis of d,l-prostaglandin B_1 (PGB_1, earlier PGE_1-278) XXX, the racemate of a naturally occurring prostaglandin and base degradation product of PGE_1 V. Reaction of ketone XXV (ethyl ester of VIII) with Grignard reagent XXVI led to the tertiary alcohol XXVII; allylic rearrangement and oxidation to ketone XXIX, partial hydrogenation of the triple bond followed by removal of the protecting groups gave d,l-PGB_1 XXX. The overall yield is surprisingly high (over 14%), partly because the end product has only one asymmetric center (at C-15), thus ruling out the formation of stereoisomers. Saturation of the triple bond in XXVIII led to d,l-PGE_1-237 (d,l-dihydro-PGB_1) XXXI.

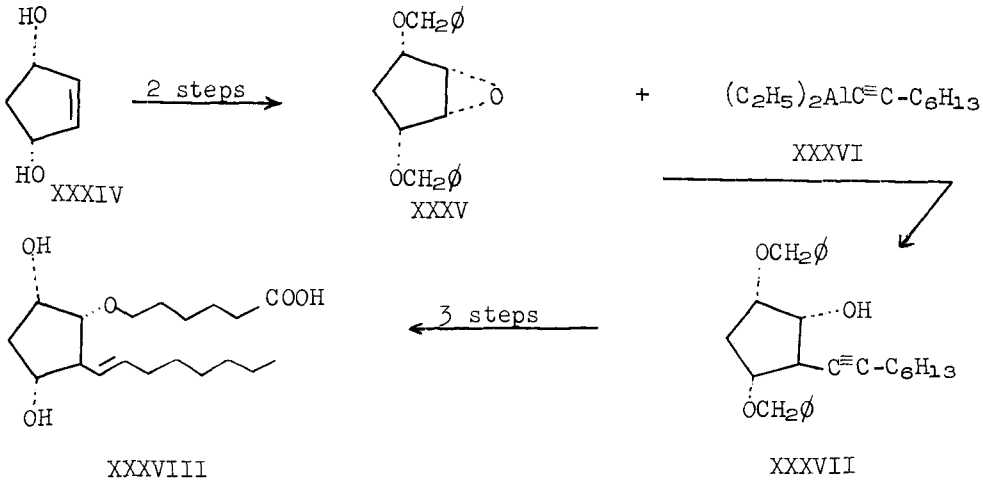


An intriguing approach was taken at Columbia University[19] based on the nitrite cleavage of bicycloketone XXXII to oxime XXXIII followed by mild conversion to d,l-PGE$_1$ V. Although intermediates potentially leading



to XXXII have been prepared, the synthesis of this key intermediate has not yet been successful.

Interesting analogs, 7-oxa-prostaglandins, have been prepared by Fried et al[20]. A key feature of this synthesis is the opening of epoxide XXXV, obtained from cis-cyclopentene-3,5-diol XXXIV, with diethyl-octynyl-alane XXXVI, which led to alcohol XXXVII. O-Alkylation with t-butyl ω-iodohexanoate, removal of the protecting groups and partial reduction of the triple bond yielded d,l-15-desoxy-7-oxaprostaglandin F$_{1\alpha}$ XXXVIII. The corresponding cis-isomer and dihydro compound were prepared by minor variations of this sequence.



In summary, a few naturally occurring prostaglandins have been prepared by non-enzymatic synthesis and some interesting analogs have been made. But so far none of these is completely satisfactory either as a practical way to these precious compounds nor in a classical sense as final structure proof of the natural product.

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Chapter 28. Nucleosides and Nucleotides

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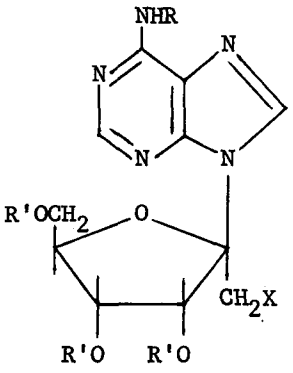
Recent discovery of a rapidly growing number and variety of nucleoside antibiotics[1], "minor nucleosides" of transfer RNA[2,3], and other naturally occurring nucleoside derivatives[4,5], as well as the promising biological activities of some synthetic nucleoside analogs in various test systems, further stimulated work in the field of nucleoside synthesis during the past year. Essentially, the methods developed in the preceding years have been employed with some modifications and refinements; no new general methods of nucleoside synthesis were reported during 1967.

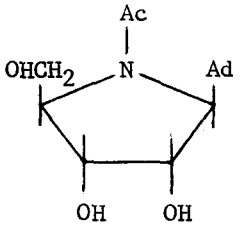
For the direct synthesis of purine nucleosides, variations of the chloromercuri method and the fusion procedure have been employed. The former method was used in the syntheses of several 2-substituted-N^6-methyl derivatives of adenosine (vasodepressor agents)[6], 2-amino-2-deoxy sugar nucleosides of adenine[7,8], and two new series of nucleosides (i.e., β-L-ribosides and β-D-3'-deoxyribosides) derived from 2-acetamido-6-chloropurine[9]; the latter compound was, however, used in the form of an unusual, incompletely characterized mercury derivative[9] rather than the chloromercuri salt. In the reaction between the bromomercuri derivative of hypoxanthine and acetobromoglucose, the 1,7 and 1,9-diglucopyranosylhypoxanthines were produced simultaneously, in considerable yields, in addition to the 7- and 9-monoglucosides[10]. In the synthesis of 9-(1-deoxy-β-D-psicofuranosyl)adenine (I), chloromercuri-6-benzamidopurine was reacted with the relatively stable 1-bromo derivative of the protected 1-deoxy-D-psicofuranosyl bromide, to give II, which was then selectively reduced to III, using tri-n-butyltin hydride in the presence of 2,2'-azobis(isobutyronitrile) as catalyst, in refluxing benzene[11].



I R = R' = X = H
II R = benzoyl, R' = p-toluoyl, X = Br
III R = benzoyl, R' = p-toluoyl, X = H

Several workers employed with apparent advantage the modification of the chloromercuri procedure involving direct use of the 1-acetates of the various blocked sugars in the presence of titanium tetrachloride. Thus, all the eight possible isomeric 9-tetrofuranosyladenines were obtained by the coupling of chloromercuri-6-benzamidopurine with the triacetates of each of the four tetroses, D- and L-erythrose and D-and L-threose, respectively, in the presence of titanium chloride, followed by deacylation and subsequent resolutions of the resulting four pairs of α- and β-nucleosides by chromatography on a

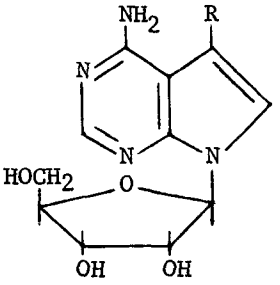
strong anion exchange resin[12]. Analogous reactions with 1-acetyl-2,3,5-tri-(O,S)-benzoyl-3-thio-D-ribofuranose[13], 1,2-di-O-acetyl-5-O-benzoyl-3-O-methyl-D-ribofuranose[14], and 4-acetamido-1,2,3,5-tetra-O-acetyl-4-deoxy-D-xylofuranose[15], respectively, yielded the corresponding blocked β-nucleosides; after deacylation, 3'-thioadenosine[13], 3'-O-methyladenosine[14], and 9-(4-acetamido-4-deoxy-β-D-xylofuranosyl)adenine[15] (IV, a pyrrolidine sugar nucleoside), were obtained.



IV

By application of the acid catalyzed fusion procedure, 2,6-dichloro-9-(2,3,4-tri-O-acetyl-β-D-riboPyranosyl)purine was prepared; deacetylation with ethanolic ammonia at room temperature resulted in concomitant nucleophilic replacement of the 6-chloro group, and subsequent catalytic dehalogenation in the 2-position gave the pyranose ring isomer of adenosine[16]. By using methylamine and dimethylamine, respectively, for deacetylation, the N^6-methyl and N^6-dimethyl derivatives of 6-amino-9-(β-D-ribopyranosyl)purine were prepared in an analogous manner[16]. It is of interest, however, that 2,6-dichloropurine (as some other purine derivatives with electron-withdrawing groups) can be condensed by fusion with fully acetylated sugars even in the absence of an acidic catalyst in vacuo[17]. Moreover, condensation of 2,6-dichloropurine with 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose was also achieved by refluxing in nitromethane solution for 1 hr (in the absence of catalyst), although in low yield[17]. On the other hand, acid catalyzed condensation of theophylline with 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose in refluxing nitromethane or anisole gave the protected N-7 nucleoside in more than 50% yield[17]. A different modification of the fusion procedure involves direct use of unsaturated sugars (glycals)[18]. Acid-catalyzed fusion of 2-chloropurine or 2,6-dichloropurine with 3,4-di-O-acetyl-D-arabinal led to the corresponding anomeric 2'-deoxy-D-ribopyranosides[18].

Extension of the fusion procedure to other heterocycles led to the total synthesis of several 7-(β-D-ribofuranosyl)pyrrolo-[2,3-d]pyrimidines[19], including the important antibiotics, toyocamycin (V) and sangivamycin (VI)[20]. The structures of these compounds have been unequivocally established; V was converted to the related antibiotic tubercidin (VII).[20]



V R = CN
VI R = $CONH_2$
VII R = H

Acid catalyzed fusion of 2-nitroimidazole (azomycin) with tetra-O-acetyl-β-D-ribofuranose at 210° for 7 minutes in vacuo gave, after deacetylation, 2-nitro-1-(β-D-ribofuranosyl)imidazole[21].

Schramm et al.[22] reported further studies and improvements on their originally poorly reproducible nucleoside synthesis which involved the condensation of unprotected sugars with the free bases by the use of polyphosphate esters. Heating a mixture of adenine and ribose, or 2-deoxyribose, with phenyl polyphosphate at 100°, for 3 min, in DMF solution containing some conc. HCl, gave mixtures of the α and β anomers of adenosine, or 2'-deoxyadenosine, respectively, in yields of 20 and 40%[22]. A new synthesis of 9-(β-D-arabinofuranosyl)-2-chloroadenine, based on the method of Glaudemans and Fletcher, employed the reaction of 2,3,5-tri-O-benzyl-α-D-arabinosyl chloride with 2,6-dichloropurine in methylene chloride in the presence of molecular sieves[23]. A new protecting group, pivaloyloxymethyl (Pom) was used for blocking of the N-7 position of adenine in the synthesis of 3-(2'-deoxy-D-ribofuranosyl) adenine; the Pom group is readily removable by mild base[24].

Several methods were reported for the synthesis of purine nucleosides from 5-amino-1-β-D-ribofuranosyl-4-imidazole carboxamide. Guanosine and its derivatives were synthesized via ring closure with benzoyl isothiocyanate[25] or sodium methyl xanthate[26]; S-methylation[25], or oxidation of the mercapto group to the sulfonic acid[26] facilitated nucleophilic displacement of the sulfur by treatment with ammonia. Ring closure with suitable ester reagents gave inosine, 2-alkylinosines and xanthosine[27], respectively. An apparently useful, general synthesis of various guanine nucleosides involves the nucleophilic displacement of the 6-chloro group in the corresponding 2-amino-6-chloropurine nucleosides (which are readily obtained via the mercury derivative of 2-acetamido-6-chloropurine) with mercaptoethanol-sodium methoxide, followed by facile hydrolysis of the intermediate 6-(2-hydroxyethylmercapto) derivatives[9].

N^6- (Δ^2-isopentenyl)adenosine (IPA)[2,28] and various other N^6-substituted adenosines[29] having cytokinin activity, were synthesized by condensation of 6-chloro-9-(β-D-ribofuranosyl)purine with the appropriate amino compound. However, a better method for the synthesis of IPA[2] or its 5'-phosphate[28] (a "minor nucleotide" of transfer RNA) involves the alkylation of adenosine or adenylic acid with α,α-dimethylallyl bromide to give the N-1 substituted intermediate which on heating undergoes rearrangement to the N^6-alkylated product[28]. A somewhat analogous, N-3 $\longrightarrow$ N^4 rearrangement occurred upon alkali treatment of methylated tubercidin[30].

A series of 8-aminopurine nucleosides and their N^8-substituted derivatives were prepared via direct bromination of the natural purine ribosides and deoxyribosides and subsequent nucleophilic displacement of the 8-bromo group with hydrazine or azide (followed by catalytic reduction), hydroxylamine or alkylamines[31]. A new synthesis of 2-fluoroadenosine was developed, starting from 2,6-diazido-9-(tri-O-acetyl-β-D-ribofuranosyl)purine which was reduced to the 2-amino-adenosine derivative; the latter was selectively diazotized in the presence of fluoroboric acid[32]. 2-Fluoro-3'-deoxyadenosine was prepared from the unprotected 2-amino-3'-deoxyadenosine in an analogous manner[33]. 2-Aminoadenosine was also prepared by Raney-nickel catalyzed reduction of 2,6-dihydroxylamino-

purine riboside[34]. Treatment of acetylated inosine-1-N-oxide with phosphoryl chloride in the presence of picoline gave the corresponding protected 2,6-dichloropurine nucleoside[35].

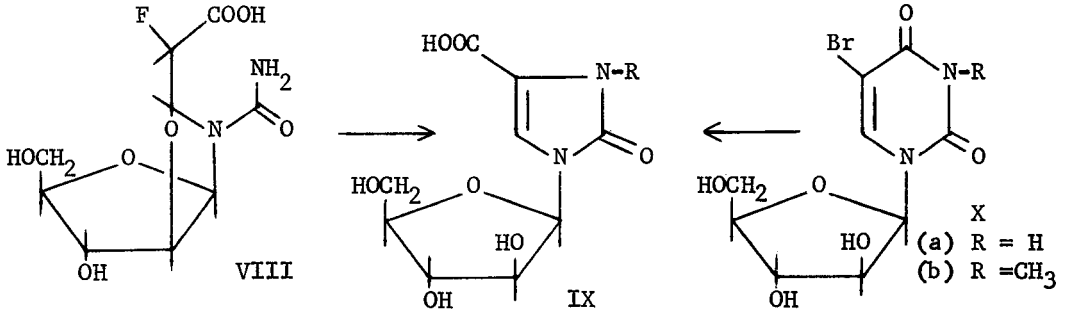
By modification of the sugar moiety, 9-(2,3-O-isopropylidene-α-D-lyxofuranosyl)adenine was obtained from the corresponding mannofuranosyladenine derivative *via* periodate oxidation followed by reduction of the 5'-aldehyde with sodium borohydride[36]. Several amino sugar nucleosides of theophylline were prepared *via* periodate oxidation of the β-D-glucoside or β-D-riboside, followed by cyclization of the dialdehyde with nitromethane and catalytic reduction of the resulting nitro sugar nucleosides[37]. Periodate oxidation of tubercidin (IV) and toyocamycin (V) followed by treatment with phenylhydrazine led to the isolation of glyoxal bisphenylosazone together with the corresponding aglycons[38]. Synthesis of the naturally occurring 2'-O-methylpurine ribonucleosides was accomplished *via* selective 2'-O-methylation with diazomethane[39].

Epoxide ring opening reactions of 9-(2,3-anhydro-5-deoxy-β-D-lyxofuranosyl)adenine and of the analogous ribofuranosyl derivative with sodium azide and benzyl mercaptide, were studied in detail[40]. Several cyclonucleosides derived from 8-hydroxy- and 8-mercapto guanine and adenine, having 8,2'-and 8,3'-O- or -S-, as well as 8,5'-S-anhydro linkages, were synthesized from the corresponding 8-bromopurine nucleosides; the latter were converted to the appropriate 2',3', or 5'-sulfonates of the 8-oxo- or 8-mercaptopurine nucleoside intermediates before or during cyclization[41-43]. Desulfurization of the 8,2'-S-cyclonucleoside derived from adenosine with Raney-nickel yielded 2'-deoxyadenosine, while desulfurization of the 8,3'-S-cyclonucleoside yielded 3'-deoxyadenosine[43]. Reaction of xanthosine with diphenyl carbonate resulted in 3,5'-cyclization of the expected 2',3'-carbonate; subsequent mild alkali treatment yielded 3,5'-cycloxanthosine[44].

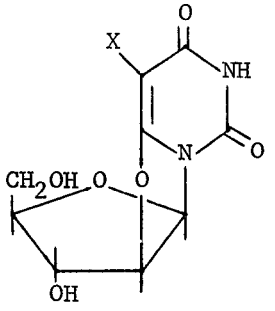
Several new pyrimidine nucleosides have been prepared by direct synthesis, but many more were obtained by chemical transformations of other pyrimidine nucleosides. 1-(2-Amino-2-deoxy-β-D-glucopyranosyl) thymine and the corresponding nucleoside of cytosine were synthesized *via* fusion of the fully protected amino sugar halide with the bis(trimethylsilyl) derivatives of thymine and cytosine, respectively[45,46,7]. As N-blocking groups for the amino sugar, 2,4-dinitrophenyl[45], trichloroacetyl[46], trifluoroacetyl[46] and bis(phenoxy)phosphinyl [$-PO(OC_6H_5)_2$][7] where employed. The latter could be readily removed by conversion to a bis(benzyloxy)phosphinyl group [$-PO(OCH_2C_6H_5)_2$] with ammoniacal benzyl alcohol at room temperature followed by hydrogenation at low pressure in the presence of palladium-charcoal catalyst[7]. Coupling of tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide with (dithyminyl)mercury[45], or, with the mercury salt of N-acetylcytosine[47], yielded exclusively[45] or predominantly[47] the corresponding O-glucoside. Failure of the latter to undergo rearrangement to the N-glucoside on treatment with mercuric bromide in xylene, was attributed to the stabilizing influence of the dinitrophenyl group on the glycosidic bond[47]. Several new 6-azapyrimidine nucleosides[48,49] and various pentopyranosyl deriva-

tives of thymine[50,51] have been prepared by direct synthesis, using modifications of the silyl[48-50] or mercury[51] methods. The Hilbert-Johnson method was recently reviewed[52].

In view of the biological activity found in nucleosides containing an arabino moiety, the synthesis and chemistry of arabinonucleosides received particular attention. Two selectively methylated derivatives of 1-β-D-arabinofuranosyluracil(spongouridine) were prepared, i.e., the 2'-O, N-3 and 3'-O, N-3 dimethyl derivatives[53]. While the former, having a blocked 2'-OH group, is relatively stable to aqueous sodium hydroxide, the latter is readily cleaved[53] to a "6,2'-anhydro," open-chain ureide, in a manner reported previously in the case of 1-β-D-arabinofuranosyl-5-fluorouracil. The unusual transformations of the latter compound were further investigated[54]. The ureide (VIII), on heating in 1.0 N sodium hydroxide, was converted to 1-(β-D-arabinofuranosyl)-2-oxo-4-imidazoline-4-carboxylic acid (IXa) which was also obtained directly, in 61% yield, from 1-β-D-arabinofuranosyl-5-bromouracil (X,a) under similar conditions; from N-3-methylated X,b, 70% yield of IX,b was obtained[54]. Iodination of



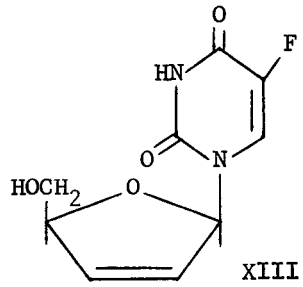
1-β-D-arabinofuranosylcytidine with iodine–iodic acid yielded, in addition to the expected 5-iodocytosine arabinoside, a product identified by spectra and X-ray crystallography as 5-iodo-O^6,2'-6-hydroxycyclouridine (XI)[55]. In the absence of alkaline conditions, an intermediate hydrogen iodide-5,6-adduct of XI could be isolated. Catalytic hydrogenation of XI yielded O^6,2'-6-hydroxycyclouridine (XII)[55]. Thiation and subsequent methylation of suitably protected 2'-deoxy-2'-(fluoro-or chloro)uridines gave the corresponding 4-methylthio-2-pyrimidone nucleosides which, by treatment with liquid ammonia yielded, in addition to the 2'-deoxy-2'-halocytidines, the halide salts of 1-β-D-arabinofuranosyl-2-amino-1,4(2H)-4-iminopyrimidine; formation of the latter product was shown to proceed via 2,2'-anhydroarabinofuranosylcytosine[56]. The reaction of various 2,2'-anhydroarabinofuranosyl pyrimidines with liquid ammonia afforded the arabinosides of 5-methylisocytosine, 5-fluoroisocytosine and 4-thioisocytosine[56]. Reaction of 2,2'-anhydro-1-(5-O-trityl-β-D-arabinofuranosyl)uracil with hydrogen sulfide in



XI X = I
XII X = H

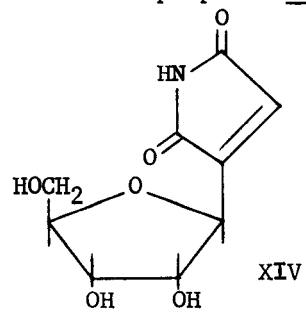
alkaline medium, followed by detritylation, yielded 1-β-D-arabinofuranosyl-2-thiouracil which, *via* thiation and subsequent amination, was converted to the arabinosides of cytosine (CA) and 2-thiocytosine[57]. The original procedure for the synthesis of CA, *via* conversion of cytidylic acid with polyphosphoric acid to a phosphorylated 2,2'-anhydrocytidine, followed by hydrolysis and dephosphorylation, was greatly improved by the application of Dekker's chromatographic separation method[58].

2',3'-Dideoxynucleosides and 2',3'-unsaturated nucleoside analogs are of interest as potential chain terminators of DNA biosynthesis. 2',3'-Dideoxycytidine and the corresponding 2',3'-unsaturated nucleoside were synthesized *via* an "epoxy" nucleoside intermediate[59]. The synthesis of 2',3'-dehydro-5-fluoro-2'-deoxyuridine (DHFUDR, XIII) was achieved by treatment of the 5'-unprotected 2,3'-anhydronucleoside with potassium *t*-butoxide in DMSO at room temperature; catalytic hydrogenation of XIII yielded 2',3'-dideoxy-5-fluorouridine[60]. While the latter showed little biological activity, DHFUDR (XIII) was a highly potent inhibitor of thymidine kinase-less (FUDR-resistant) Novikoff hepatoma cells in culture. *In vivo*, DHFUDR showed high activity against Sarcoma 180, leukemia L1210 and various FUDR-sensitive and FUDR-resistant transplanted leukemias in mice[60]. Other new, biologically active 5-fluoropyrimidines were prepared by the addition of methyl hypobromite across the 5,6-double bond; the resulting 5,6-substituted 5-fluoro-5,6-dihydropyrimidines and their 2'-deoxyribonucleosides showed *in vivo* activity, apparently acting as releasers of 5-fluorouracil (FU) and 5-fluoro-2'-deoxyuridine(FUDR), respectively[61].



XIII

Interesting "imino-bridge" analogs of 2,3'-anhydrothymidine were synthesized by treatment of 3'-O-mesyl-2,5'-anhydrothymidine with liquid ammonia or methylamine[62], and a pyranoside analog of 2,3'-anhydrothymidine was also reported[63]. Uridine was converted to its C_4'-epimer, 1-α-L-lyxofuranosyluracil[64], and several pyrimidine α-L-lyxo and α-L-xylonucleosides were prepared *via* the directly synthesized α-L-arabinosides[65]. The metaperiodate-nitromethane procedure was utilized for the synthesis of 3'-nitro- and 3'-amino-3'-deoxyglucopyranosides of cytosine[66] and thymidine[67].



XIV

Various nucleosides of 5,5-disubstituted barbituric acid[68] and 4-alkoxy-6(1H)-pyrimidones[69] have been reported. Several indole nucleosides were synthesized[70], and a review was published on imidazole nucleosides and nucleotides[71]. The structure of the antibiotic showdomycin XIV was established as 2-(β-D-ribofuranosyl)maleimide, *i.e.*, a "C-nucleoside" related to uridine[72,73]. Two other "C-nucleosides", 1-deazuridine and 2'-deoxy-1-deazauridine, were synthesized analogously to pseudouridine; however, these compounds were highly labile[74].

In the synthesis of nucleotides, β-cyanoethylphosphoric acid with DCC in pyridine remained the most widely employed phosphorylating agent[75]. However, a reinvestigation of the use of phosphoryl chloride resulted in an apparently very useful, novel method for the synthesis of nucleoside-5'-phosphates[76]. Treatment of the 2',3'-O-isopropylidene-protected nucleosides with phosphoryl chloride in a trialkyl phosphate solvent, at low temperature, followed by hydrolysis of the phosphochloridates and deprotection, gave nearly quantitative yields of the 5'-nucleotides. Moreover, the latter could be obtained in 80-90% yields even from the unprotected nucleosides by the same procedure if the phosphorylating agent was pretreated with a small amount of water[76]. A direct synthesis of purine nucleotides was also reported, involving the fusion of fully acetylated sugar-5'-phosphates with 9-acetylpurines at 150°, to give a mixture of the anomeric nucleoside-5'-phosphates and of the corresponding diphosphates[77].

Several structural analogs of the nucleoside-5'-monophosphates have been synthesized in which the phosphate moiety is replaced by sulfate[78], nitrate[79], phosphite[80], or methanephosphonate[81]. Structurally most similar to the 5'-nucleotides are the corresponding 5'-deoxynucleoside-5'-phosphonic acids[82,83]. Further studies, concerning the potential biological activities of such compounds, should be awaited with interest.

Various protecting groups have recently been adopted in the synthesis of mono- and/or oligonucleotides. For the protection of cis-(2',3')-diol systems, *in lieu* of the isopropylidene group, recently the more acid-labile methoxy- or ethoxymethylidene, methoxyethylidene and methoxybenzylidene groups have been increasingly employed; such blocking groups are introduced *via* acid-catalyzed orthoester exchange reactions[84]. For the protection of the 2'-, or both the 2'- and 5'-hydroxyls, *in lieu* of the tetrahydropyranyl group[85,86] which leads to diastereoisomers[86], the symmetrical 4-methoxytetrahydropyranyl group has been proposed[87]. The β-benzoylpropionyl [C_6H_5-CO-CH_2CH_2-CO-], a "blocking group with a trigger for selective cleavage," was introduced recently for the protection of the 3'-hydroxyl in Letsinger's oligonucleotide synthesis.[88] This group is stable to either acid or alkali, but is removable after reaction with hydrazine under essentially neutral conditions[88]. The interesting adamantoyl group was also proposed for protection of the 5'-hydroxyl[89].

Numerous dinucleoside phosphates, di- and trinucleotides have been synthesized containing an "unnatural" nucleoside or nucleotide, such as cytosine arabinoside (CA)[90,91], 6-azauridine[92,93], 5-halo-2'-deoxyuridines[93], 5'-deoxyuridine-5'-phosphonate[94], thymidine-5'-phosphorothioate[95]. Biological studies on the CA-derivatives indicate that their activities in cellular systems depend on cleavage with the release of CA[96,97].

Several modifications of Letsinger's procedure[88,98,99] and two novel procedures[100,101] were reported for the synthesis of oligonucleotides on insoluble polymer supports. Khorana's procedure, using soluble polymer support, was published in detail[102].

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Chapter 29. STEROIDS

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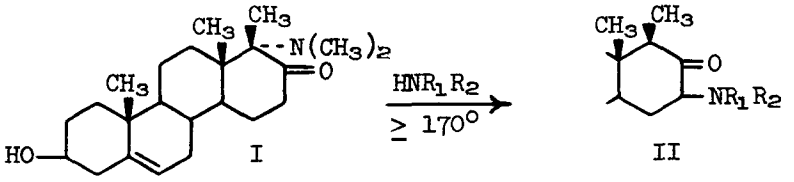
Introduction - The year saw an explosion in the number of publications on the total synthesis of steroids, and especially estratriene derivatives. Research in the field of steroidal alkaloids is also reaching monumental proportions and is fast becoming the single most active field of steroid chemistry. For example, recent work by Kyoto chemists has led to the isolation and identification of 27 pregnane alkaloids from the common Pachisandra Terminalis.[1]

General - Preliminary reports on a new fragmentation of α,β-epoxy ketones to acetylenes and carbonyl-containing products showed that the reaction can be of general application and a number of variations have already been described.[2,3] Thus, for the first time it has been possible to prepare readily secosteroid acetylenic ketones.

Tosylhydrazones on reaction with alkylithium reagents give olefins in high yield. This new synthesis allowed for example the preparation of 16-androstene from androstan-17-one in quantitative yield.[4,5] Reduction of carbonyl functions to methylene can be effected electrochemically[6] or by treatment of the steroid ketones with powdered zinc in acetic anhydride saturated with hydrogen chloride at room temperature.[7]

A convenient method for transposing the oxygen of a carbonyl to an adjacent methylene involved condensing the ketone with an aromatic aldehyde, reduction of the resulting arylidene ketone with lithium aluminum hydride-aluminum chloride reagent to the corresponding desoxy compound, followed by ozonolysis to the new ketone. This way a 3-ketone led to a 2-ketone[8] and a 17-ketone was converted to a 16-ketone.[9] 17α-Bromoethynyl and 17α-iodoethynyl steroids can be made by metal-halogen interconversion using respectively bromotrifluoromethane and iodoheptafluoropropane on the appropriate lithium acetylide derivatives.[10] The synthesis of steroids having the novel 3-deoxy-1,4-diene structure has been described. It involved step-wise reduction of a $\Delta^{1,4}$-3-ketone first with lithium aluminum hydride followed by lithium-in-ammonia hydrogenolysis of the resulting mixture of epimeric 3-alcohols.[11]

Androstane Series - A new type of substitution reaction has been described. When the D-homo steroid (I) was treated with a primary amine, attack occurred at the 16 position with elimination of dimethylamine at 17a, to yield II.[12] Most probably the key step is a SN2' displacement on the enol by the entering amino group.



I II

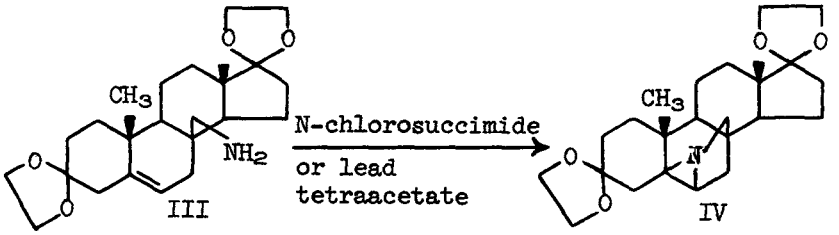
Additional examples of backbone rearrangement of steroids have been described.[13,14,15]

Derivatives of 17β-hydroxy-19-norandrosta-4,9-dien-17-one and 17β-hydroxy-19-norandrosta-4,9,11-trien-17-one have been the subject of extensive studies by various groups because of their marked anabolic and androgenic activities.[16,17,18] The 2-oxa analogs have also been prepared.[19]

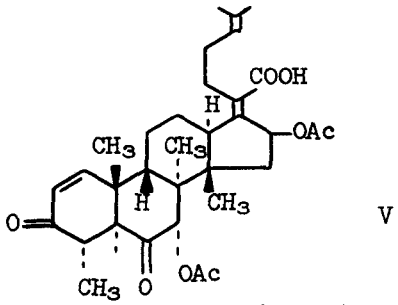
An acid catalyzed rearrangement product of 3α,5α-cyclo-6,19-dioxo-17β-acetoxy-androstane has been converted to 19-nor-A-homotestosterone.[20] This compound has λ_{max} 243 mμ, whereas A-homotestosterone shows λ_{max} 235 mμ. The unexpected shift in the ultraviolet absorption could be attributed to a difference in the respective conformations of ring A. This type of problem has been studied recently.[21]

Rigorous experimental analysis of the factors influencing the relative stability of C/D trans versus C/D cis 17-alkyl-15-oxo androstanes has shown that as the size of 17-alkyl group increases, the amount of C/D trans product predominates over the C/D cis isomer at equilibrium.[22]

A novel synthesis of bridged aziridines has been discovered by the Shionogi chemists. The reaction is quite general and involves an intramolecular addition of a nitrene to a double bond, as exemplified by the high yield conversion of III to IV.[23]



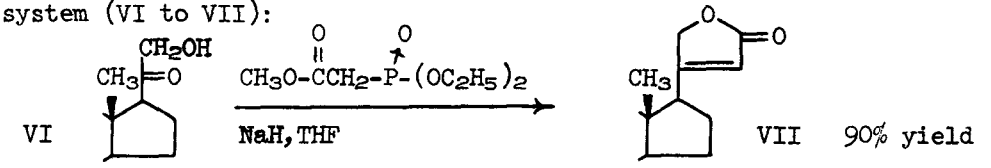
Antibiotics - The complete structure V for Helvolic acid has been proposed, based on chemical evidence.[24]



V

Cardenolides and Related Structures - A number of new synthetic derivatives of strophanthidin have been prepared in an attempt to delineate and compare the structural requirement for activity.[25] Other modified cardenolides having C_{19} halogens have been prepared.[26] Two novel methods for the preparation of unsatured lactones have been developed and the

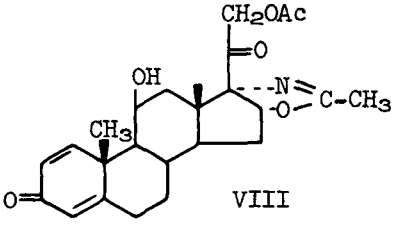
most elegant one involved the condensation in the presence of sodium hydride of diethyl methoxycarbonylmethylphosphonate[27] with an α-ketol system (VI to VII):



The Rehovoth group has discovered that cardenolides can react with selenium dioxide to form 17α-hydroxy derivatives in relatively good yields.[28]

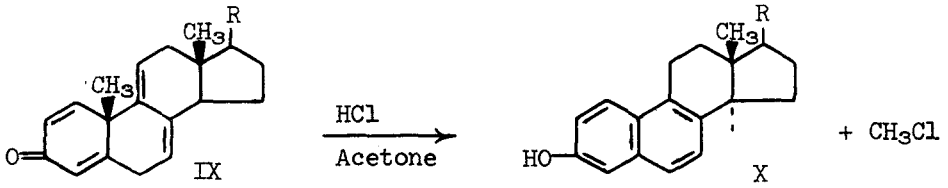
The optical rotatory dispersion of a large number of cardenolides has been studied. They showed a distinct Cotton effect caused by the butenolide ring and extrema at 228 mμ and 260 mμ were observed.[29]

Corticosteroids - Some attention has been directed towards finding better methods for esterification of the various hydroxy groups. Thus, when cortisol was treated with formic acid in the presence of phosphoric anhydride, the 11β,17α,21-triformate derivative was obtained.[30] Similarly, when the complex $(CH_3CO)^+SbCl_6^-$ was used the corresponding triacetate was formed.[31] Reaction of C-21 hydroxylated steroids with pyrophosphoryl chloride led to a convenient synthesis of 21-phosphate esters.[32] It is interesting to note that the (17α,16α-d) oxazolino grouping is compatible with activity. For instance, VIII had 35 times the activity of cortisol in the anti-granuloma assay.[33]



A clever approach has been designed for the preparation of 16α,17α-dimethyl corticoid, involving conjugate 1,4-addition of methyl magnesium iodide to the Δ^{16}-20-ketone system, followed by methylation of the resulting magnesium enolate with methyl iodide.[34] On the other hand, attempted conjugate 1,4-addition of methyl magnesium iodide to prednisone-BMD gave 40% of the expected 1α-methyl cortisone-BMD and 35% of the 3-methylene derivative arising from a 1,2-addition.[35]

Estratriene Series - The study of the aromatization of various $\Delta^{1,4}$-3-keto steroids is continuing. Reductive aromatization of 17-hydroxyandrosta-1,4,11-trien-12-one in the presence of zinc in pyridine, proceeded normally.[36] Similarly 11-hydroxyestrone has been prepared from androsta-1,4-diene-3,11,17-trione by reduction with lithium-biphenyl.[37] Along different lines, a certain group of steroidal dienones has been shown to undergo ionic aromatization[38] as exemplified by the conversion of IX to X in the presence of hydrochloric acid:



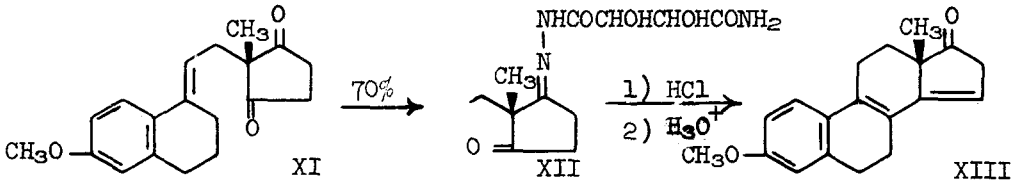
The Birch reduction of estrone gave a 77% yield of estr-5(10)-ene-3α,17β-diol.[39] Surprisingly, this same compound was obtained as the major product from the photochemical sodium borohydride reduction of estrone.[40]

The synthesis of highly potent 7α-methylestratriene derivatives has been described.[41] It is interesting to note that methylation of Δ^{16}-15-ketones gave the corresponding 14α-methyl estratriene derivative (C/D trans).[42] D-Norestratrienes have been synthesized and their chemistry studied.[43] For instance, 3-methoxy-17β-amino-1,3,5-estratriene rearranges to C-homo-D-bisnor steroids on treatment with nitrous acid.[44]

Among new compounds of interest one should mention 1,11-iminoestrones,[45] 16-oxa and 16-azaestrone methyl ether.[46]

Total Synthesis - A major effort was devoted to developing and improving the original Smith-Torgov reaction sequence leading to estrapentaenes of type XIII.

The Roussel group has achieved the first totally chemical asymmetric synthesis of a steroid. It consists in reacting the secodione XI with L-tartaric amide hydrazide under conditions where the monohydrazone XII formed was insoluble. After ring closure and hydrolysis the resolved pentaene XIII was obtained in 30% yield overall from XI.[47]



The Schering A.G. group has published full details of its asymmetric synthesis by microbiological reduction of compounds of type XI to the corresponding optically active 17-hydroxy derivatives.[48]

Other applications of the Smith-Torgov reaction involved the synthesis of 3-dialkylamino estrapentaenes,[49] aromatic C-ring analogs of 18-norestrone,[50] estrahexaenes,[51] 8α-estranes,[52] 11-oxygenated estratrienes,[53] 8α-B-norestrones,[54] 13-phenyl and 13-benzyl estrapentaenes,[55] 16-oxaestratrienes,[56] bisdehydro-doisynolic acids,[57] and A-nor-2,3-diazasteroids.[58]

A different but also elegant approach led to dl-8-azaestrone methyl ether[59] and dl-18-nor-D-homo-8-azaestrone methyl ether.[60] Also synthesized were 8,13-diaza-18-norestrone methyl ethers[61] and 8-aza-19-norprogesterone.[62] A new method using the isoxazole annelation and having the

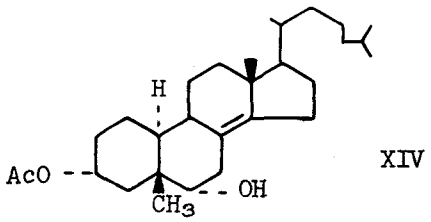
potential of wide application has been described for the total synthesis of dl-D-homotestosterone and dl-progesterone.[63]

Pregnane Series - The synthesis of 1,2-methylene 19-nor-17α-acetoxyprogesterones has been the subject of a communication.[64] It is of interest to note that the reaction of hydrazoic acid on the 16β,17β-epoxypregnenolone acetate can lead to ring opening with inversion at 16 and 17 to form the corresponding 16α-hydroxy-17α-azido derivative.[65]

A synthetic approach to the 9(10→19) abeo-9(11)-pregnene system, involved a Wolff-Kishner reduction of a 9β,10β-methylene-11-ketone.[66]

The novel preparation of 4,5-dihydro-18-hydroxyprogesterone from conessine has been reported in detail and involves the reaction of p-nitroperbenzoic acid with a 20-imine.[67]

Sterols and Related Products - The Westphalen rearrangement of some 3β-substituted-6β-acetoxy-5α-hydroxycholestanes has been studied and the products and kinetics shown to be dependent on the nature of the 3-substituent.[68] The conformations of the products were studied and in the case of the Westphalen-Lettré derivatives it was shown that ring B is in the boat conformation.[69] The boron trifluoride catalyzed cleavage of 3α-acetoxy-4α,5-epoxy-5α-cholestane afforded the expected backbone rearrangement products containing a 13(17) double bond.[70] A similar experiment with 3α-acetoxy-5,6α-epoxy-5α-cholestane yielded some of the $\Delta^{8(14)}$ rearranged derivative XIV after a reaction time of 25 seconds, along with the expected $\Delta^{13(17)}$ isomer:[71]

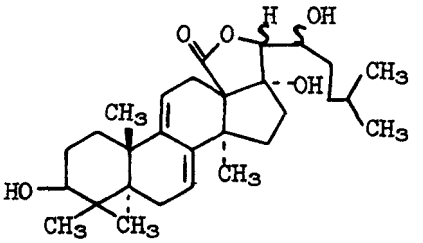


XIV

Base catalyzed decomposition of cholesteryl acetate ozonide led in one step to the corresponding seco-acid in 70% yield, thus making this type of system readily available.[72] Cholestanone dimethyl ketal, on reaction with acetamide in the presence of acid, gave 3-acetamido-2-cholestene.[73]

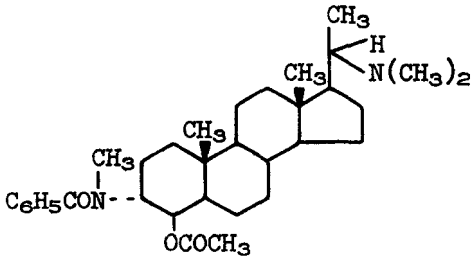
Using labeled mevalonic acid it was proved that cholesterol is not an intermediate in the biosynthesis of tigogenin.[74] A formal total synthesis of tachysterol$_3$ has been claimed.[75]

The new triterpenoid sapogenin griseogenin of the sea cucumber has been identified as 22-hydroxyhalothurinogenin[76] (XV).



XV

Steroidal Alkaloids - Plant steroids with 21 carbon atoms have been reviewed.[77] As mentioned in the introduction, the Pachisandra alkaloids were subjected to a thorough and meticulous study. Without exception all the 27 alkaloids isolated so far were identified as belonging to the pregnane group and were related to pachisandrine-A (XVI). It is interesting to note that some of the alkaloids extracted from the South American species Malouetia arborea and Malouetia tamaquarina possess the conessine ring system.[78]



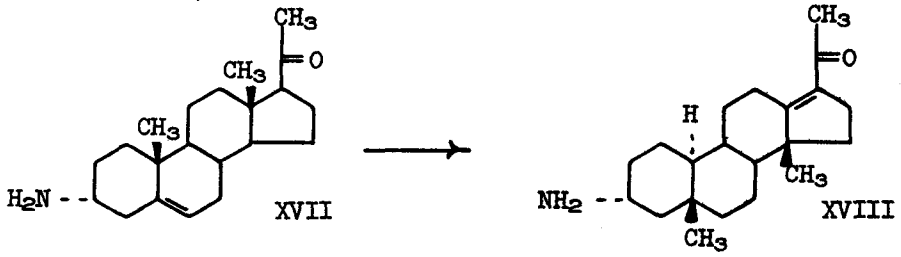
XVI

Verazine is a new veratrum alkaloid derived from 22,26-iminocholestane.[79] Veralkamine from Veratrum album, a novel type of steroidal alkaloid, has a 17β-methyl-18-nor-17-isocholestane carbon skeleton. The complete structure was determined by x-ray crystallography.[80] A complete x-ray structural analysis of tomatidine hydroiodide showed that the spiro carbon at 22 has the S-configuration and that the C_{25} methyl is equatorial.[81] The configuration of veratramine and jervine have been revised: the C_{22} and C_{25} substituents were shown to be trans to each other.[82]

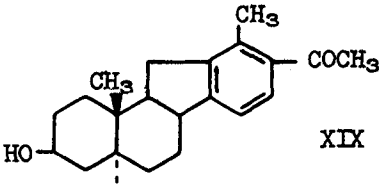
In connection with the preparation of various aminosteroids, the influence of the nature of solvent on the azidolysis of various tosylates was investigated. In the case of 3β-tosyloxy-Δ^5 steroids, the highest yield of 3α-azido-Δ^5 products was obtained with hexamethylphosphoramide. It was also the solvent of choice in the case of 20β-tosylates.[83] The rearrangement of amine oxides in the presence of anhydrides (Polonovski reaction) was investigated on N-methyl-5α-dihydroparavallarine, a 3β-dimethylaminosteroid. It was found that the course of the reaction could be changed depending on the anhydride used. Acetic anhydride led to the 3-acetylmethylamino derivative in 70% yield, whereas trifluoroacetic anhydride, after mild basic hydrolysis, afforded the corresponding 3-ketone in 60% yield and the 3-methylamino derivative in 40% yield.[84]

It has been known that dissolution of conessine in cold sulfuric acid-acetic acid mixture gave rise to rearrangement of the molecule. A new product, novoconessine, was isolated recently from this reaction

mixture. It is the 10α(H)-epimer of neoconessine.[85] On the other hand, dissolution of 3α-amino-5-pregnen-20-one (XVII) in cold sulfuric acid led to a backbone rearrangement to give the A/B trans unsaturated ketone XVIII. It is important to note the 3β-dimethylamino analog afforded the corresponding A/B cis derivative.[86]



The 17β-hydroxy positional isomer of samandarine was synthesized starting from 17β-acetoxy-1-androsten-3-one.[87] A formal partial synthesis of samandarone was announced using testosterone as a relay. The last step of this synthesis consisted in transposing the 17-oxygen to the 16 position.[88] The total synthesis of 17-acetyl-5α-etiojerva-12,14,16-trien-3β-ol (XIX) was accomplished recently by the Stanford group.[89] This work led to a formal total synthesis of Veratramine[90] and Jervine[91] using XIX as a relay.



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Chapter 30. Reactions of Interest in Medicinal Chemistry

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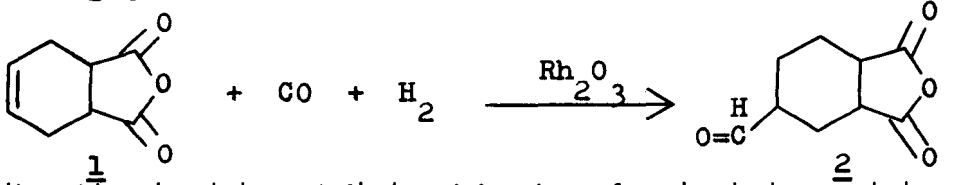
The seemingly endless procession of useful new chemical reactions and techniques appearing in the literature is a constant wonder to the bibliographer and a source of comfort to the medicinal chemist who is faced with ever more sophisticated synthetic challenges. This narrative is intended to be illustrative rather than thorough or exhaustive.

Alkynes in ethereal solvents are stereospecifically reduced by $LiAlH_4$ to trans-olefins[1]. This result contrasts with the cis-products reported from $LiAlH_4$ reduction of alkynes in hydrocarbon solvents. A homogeneous catalytic hydrogenation of unsaturated aldehydes to saturated aldehydes can be accomplished by use of a tris-(triphenylphosphine)chlororhodium catalyst[2]. The reaction is specific for carbon-carbon multiple bonds, and other functional groups do not interfere. Use of dilute reaction solutions minimizes decarbonylation of the aldehyde. Reaction of $NiCl_2$ with methyl hydropolysiloxane gives rise to a black substance which is an extremely active catalyst for hydrogenation of carbon-carbon double bonds[3]. The catalyst is inactivated by diethyl ether or tetrahydrofuran, but it can be reactivated by addition of a large excess of water. A complex cobalt dihydride is formed by reaction of cobalt stearate with alkyl Grignard reagents[4]. In addition to having catalytic ability, the compound stoichiometrically hydrogenates alkenes to alkanes. Olefinic acids and derivatives can be hydroformylated with CO and H_2 in the presence of Rh_2O_3[5]:



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Acrylic acid analogs behave similarly, giving rise to formyl or hydroxymethyl systems, hydroxypivalates, and γ-butyrolactones[6].

A $NaBH_4$-cobalt(II)-α,α'-bipyridyl system reduces azidosteroids to amines under conditions which $NaBH_4$ alone is ineffective[7]. Earlier workers had found that this system is stronger than $NaBH_4$ for reduction of nitrobenzene to aniline.

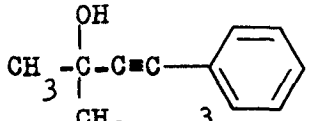
Ag_2O in acidic media converts primary alcohols to aldehydes and secondary

alcohols to ketones under mild conditions[9]. The reaction appears to be specific for alcohol groups, and it succeeds in instances where tetravalent cerium reagents fail. Argentic picolinate has been reported to have a spectrum of oxidizing ability similar to Ag_2O[10]. In addition, however, the picolinate salt converts amines to the corresponding carbonyl system. Significant utility of this reagent in carbohydrate chemistry is suggested. A rapid preparative method with minimal explosion hazard for $AgClO_4$ and $AgBF_4$ involves precipitation from ethylbenzene[11].

Aliphatic and alicyclic olefins are oxidized to ketones by a pertrifluoroacetic acid-BF_3 reagent[12]. For some carbocyclic systems ring contraction, expansion, or rearrangement may also occur, forming interesting compounds not otherwise readily preparable. An unusually effective oxidizing form of MnO_2 results from ozonization of $Mn(NO_3)_2$[13]. This reagent converts cinnamyl alcohol to cinnamaldehyde in 94% yield and triphenylphosphine to triphenylphosphine oxide in 68% yield.

A facile conversion of several aliphatic and aromatic acyl peroxides, halides, and anhydrides to the "per" acids has been effected by treatment with H_2O_2 and a threefold excess of base in the presence of a soluble magnesium salt[14]. The magnesium salt is proposed to stabilize the "per" acid as it is formed, and to prevent its decomposition.

Grignard reagents of the types CX_3MgCl, CHX_2MgCl, and CH_2XMgCl (X=halogen) have been reported[15]. The latter two structures show little carbenoid character, but CX_3MgCl reacts with olefins to form dihalocyclopropanes in poor yields. All three of the systems react as normal Grignard reagents with acid anhydrides and aldehydes, but they do not react with esters or ketones. Phenylethynylmagnesium bromide reacts with propylene oxide to give a 90% yield of 3 and only trace amounts of the "normal" product 4 expected of propylene oxide ring opening due to attack on the primary carbon[16].



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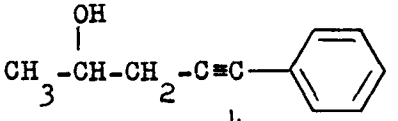

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Methylenemagnesium iodide, $CH_2(MgI)_2$, prepared from magnesium amalgam and methylene iodide, reacts with aldehydes and ketones in a Wittig-like reaction, substituting a methylene group for a carbonyl[17]; this finding refutes earlier reports that gem-dimetallic systems exhibit very low reactivity toward aldehydes and ketones.

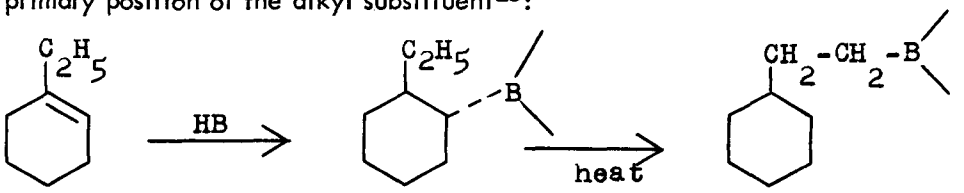
Terminal acetylenic groups can be protected from participating in undesirable reactions with organometallic reagents by first forming the ethynyl Grignard reagent and treating it with trimethylchlorosilane, forming a carbon-silicon bond at the primary acetylenic carbon[18]. p-Bromotolane, protected by trimethylsilylation, was converted to its Grignard reagent and this was carbonated to form a benzoic acid derivative. The carbon-silicon bond was cleaved with dilute base to regenerate the

primary acetylene. Non-terminal carbon-carbon triple bonds in poly-ynes can be selectively reduced catalytically when the terminal acetylenic bond is masked by trimethylsilylation[19]. Desilylation involves hydroalcoholic $AgNO_3$ which forms the silver acetylide which is converted to the free acetylene with cyanide ion.

Trialkyl boranes react with CO under convenient laboratory conditions to form (depending upon relative amounts of reagents and reaction conditions) trialkylcarbinols, symmetrical ketones, or methylols[20]. The reaction at atmospheric pressure is greatly enhanced by the presence of lithium trimethoxyaluminohydride[21]. Some modification of the technique permits use of the reaction for conversion of an olefin to the saturated acid having one more carbon[22]; methyl undecylenate is converted to the C_{12} dicarboxylic acid, and undec-10-en-1-ol forms 12-hydroxyduodecanoic acid. Acrolein reacts with organoboranes derived from olefins to produce saturated aldehydes having three more carbons than the starting olefin[23]. The reaction is generally complete in five minutes at room temperature. Methylvinyl ketone may be similarly hydroborated[24]:

$$R_3B + CH_2{=}CH{-}\overset{O}{\overset{\|}{C}}{-}CH_3 \rightarrow R{-}CH_2{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}OBR_2 \rightarrow RCH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}CH_3$$

Both steps are complete in a few minutes at room temperature; the reaction seems quite general. Since the methyl ketones will undergo a haloform reaction, a way is available for preparation of carboxylic acids having lengthened the chain by three carbons. 1-Alkylcyclohexenes which are hydroborated normally will (as was shown some years ago in acyclic systems) isomerize with heat such that the boron migrates to the primary position of the alkyl substituent[25]:

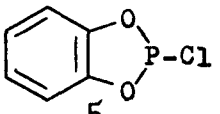


Aliphatic tosyl hydrazones containing α-hydrogen react with alkyllithium to form olefins which might otherwise be difficult to prepare[26]. Camphor tosylhydrazone was converted quantitatively into 2-bornene. The reaction appears to be general. Several 1-alkenes reacted with a lithium dispersion to form the corresponding 1-alkynyllithium[27]; attempts to extend the reaction to olefins other than 1-alkenes failed. A method has been devised[28] for preparation in high yields of methoxymethyllithium and for its manipulation in normal organometallic reactions. A 10:3 ratio of potassium tert-butoxide: water in aprotic solvents is an exceptionally mild reagent for cleavage of non-enolizable ketones[29], and causes only a slight degree of epimerization of the acidic product. Magnesium adducts of naphthalene, biphenyl, and phenanthrene are useful in metallation of fluorene, indene, and phenylacetonitrile[30]. In a somewhat analogous reaction, lithium naphthalene reacted with ethylvinyl ether to yield 1-ethylnaphthalene[31]. 2-Pyridyllithium reacts with cinchoninic acids to

form pyridyl ketones which may be reduced with $NaBH_4$ to 2-pyridyl quinoline-methanols, thence catalytically to 2-piperidyl quinolinemethanols[32].

tris-(Disubstituted amino) arsines react with aldehydes and ketones to form aminals[33]. If the original carbonyl compound bears a hydrogen on the α-carbon, the aminal will spontaneously lose the elements of a secondary amine and will form an enamine. The aminoarsine derivatives are easily prepared.

Earlier technical difficulties in the use of phosphite esters have been obviated by use of o-phenylenedioxyphosphorus chloride 5, which reacts with alcohols to form o-phenylene phosphites which upon treatment with I_2 form iodides in almost quantitative yields with little tendency for elimination to occur[34].



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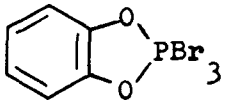

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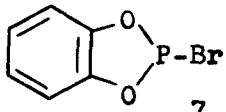

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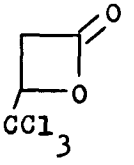
o-Phenylenedioxyphosphorus tribromide 6 converts carboxylic acids or anhydrides to the acyl bromides under mild conditions[35]. Alternately, o-phenylenedioxyphosphorus bromide 7 and Br_2 may be employed.

Carbon-carbon bonds can be formed between unlike groups by reaction of π-allyl nickel derivatives (formed from nickel carbonyl) and alkyl halides[36].

α-Bromoketones lose Br_2 to form zinc enolates which react with electrophiles to form carbon-carbon bonds at the original site of the bromine[37]. 2-Bromo-1-tetralone was converted to 2-methyl-1-tetralone in 85% yield. A variety of allyl alcohols reacts with cyclohexyl isocyanide under cuprous ion catalysis to form allyl formimidates[38].

Terminal olefins may be converted to the α,β-unsaturated acid bearing one more carbon by hydroalumination followed by treatment with CO_2[39]. Hydroalumination of disubstituted acetylenes followed by carbonation can be controlled so as to produce isomerically pure cis- or trans-α,β-unsaturated acids[39].

Formyl carbene is preparable by a copper acetylacetonate-catalyzed decomposition of diazoacetaldehyde[40]. This new carbenoid species formed a cyclopropanecarboxaldehyde with a substituted olefin. A novel approach to β-lactones of above 70% optical purity involves reaction of a carbonyl compound with a ketene (or with an acyl halide convertible to a ketene) in the presence of an optically active base[41]. Trichloroacetaldehyde reacts with ketene in the presence of brucine to form (+)-8; in the presence of (-)-1-phenyl-1-dimethylaminoethane, (-)-8 is formed.



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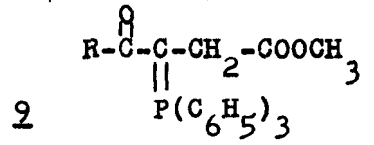
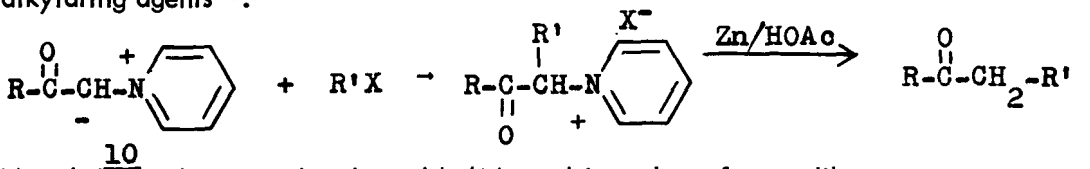
A variation of the Wittig reaction between triphenylphosphine acyl carbene and bromoacetic ester results in C-alkylation and ylide formation to give structure 9[42]. Treatment of these ylides with aqueous base results in γ-keto acids; pyrolysis of the ylides forms α,β-olefinic-γ-keto esters.



9

Dimethylsulfoxonium methylide addition to olefins, forming cyclopropane systems, has been demonstrated[43], and appears to be superior to the older method of Simmons and Smith. Stereochemistry of the cyclopropane products depends upon that of the starting olefins, but it is sometimes difficult to predict; however, the product frequently consists of only one isomer (cis- or trans-). gem-Dichlorocyclopropanes bearing an ether link elsewhere on the ring may be reductively dehalogenated with Na in liquid NH_3 without ring cleavage[44]. Pyridinium-, quinolinium-, and isoquinolinium ylides of type 10 have preparative value in their reactions with alkylating agents[45]:



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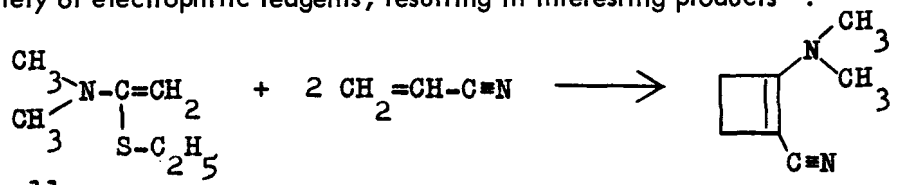
The ylide can be treated with acyl halides, ultimately to form β-diketones or β-ketoesters.

gem-Dicarboxylic esters when heated with NaCN in dimethyl sulfoxide decarboxylate to the monocarboxylic ester in high yields[46]. Since gem-dicarboxylic esters are frequently readily available, this is potentially a valuable procedure.

1-Dimethylamino-1-ethylthioethylene (ketene-N,S-acetal) 11 reacts with a variety of electrophilic reagents, resulting in interesting products[47]:



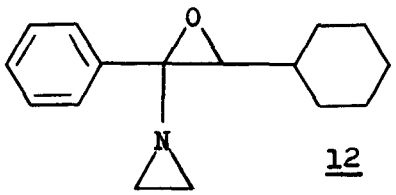
11

Iodine isocyanate reacts with olefins to form α-iodoisocyanates which can be converted to a variety of useful derivatives[48]. The method can be employed for stereospecific synthesis of cis-β-aminoalcohols. Iodine azide, prepared in situ, adds in a highly stereospecific manner to a variety of unsaturated systems[49]. Cyclohexene and cholestene form the trans- and trans-diaxial 1-azido-2-iodo systems respectively. Open chain cis- and trans- olefins yield the threo- and erythro-1-azido-2-iodo systems.

Primary and secondary amines and certain hydrazines are cyanomethylated by cyanomethylbenzenesulfonate and p-toluenesulfonate to give aminoacetonitrile derivatives[50]. Trimethyl- and triethylamines were quaternized, but other less reactive tertiary amines did not react.

Primary amines are conveniently masked by conversion to their tert-butoxycarbonyl derivatives with tert-butylcarbonic diethylphosphoric anhydride[51]. The free amine may be regenerated with KOH. A variety of tertiary amines is quaternized by refluxing with methyl salicylate[52]; benzyl and ethyl salicylates provide less satisfactory reactions. β,β,β-Trichloroethoxycarbonyl chloride acylates a variety of aromatic and aliphatic OH and NH_2 groups under mild conditions[53]. The acylated derivatives are inert toward Jones or Sarrett oxidations; dioxane-HCl; trifluoroacetic acid; and toward catalytic hydrogenation at room temperature. Treatment with Zn-acetic acid regenerates the OH or NH_2 group.

The first stable, crystalline epoxyamine 12 has been reported[54]; this versatile new functional group undergoes a variety of reactions leading to α-hydroxyketones, α-hydroxy-N-ethylamines, 1-aziridinocycloheptanones, and aziridinomethyl cyclohexanols.



12

Phenyl chloroformate is claimed to be superior to the benzyl or ethyl esters as a general reagent for cleavage of tertiary amines, comparing favorably to Br-CN in efficacy as well as convenience[55]. Sulfonamides are rapidly cleaved by sodium naphthalene anion radical; the ease of N-alkylation of sulfonamides makes possible a new method for attaining very pure secondary amines[56]. Methylcyclohexane may be directly aminated with trichloroamine in the presence of $AlCl_3$, forming 1-methyl-1-aminocyclohexane; this is a new useful synthesis for carbinamines[57].

Primary amines are converted to nitriles by lead tetraacetate[58]; however, some side products are formed.

N-Chloro-N-cyclohexylbenzenesulfonamide in the presence of a small amount of benzoyl peroxide is an excellent agent for effecting allylic chlorination[59].

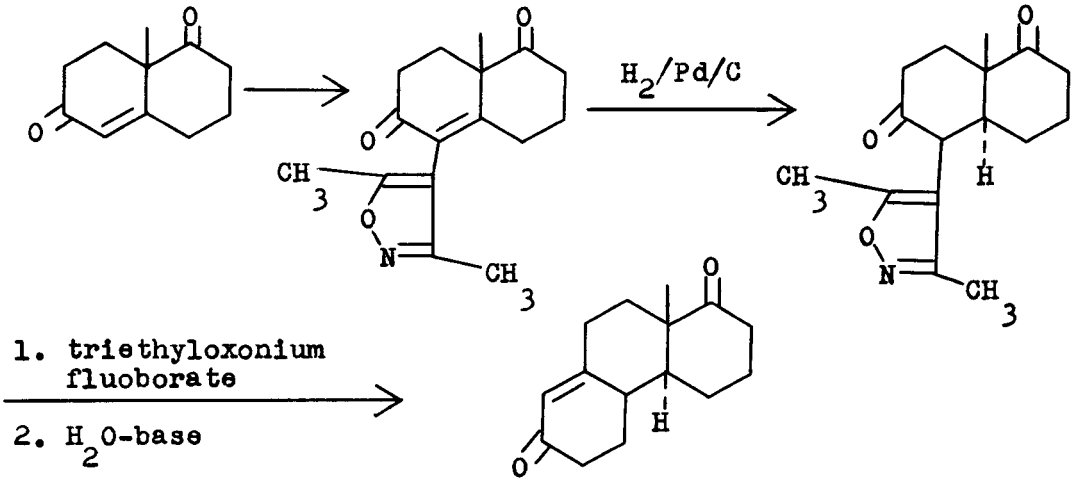
Preparation of 1,2-disubstituted adamantanes by an insertion reaction on 1-substituted adamantane systems has been reported[60]. A subsequent publication[61] describes preparation of 1-(β-hydroxyethyl)-2-hydroxyadamantane and a number of chemical transformations on this system.

The efficacy of alcoholic solutions of urea in neutralizing strong acids has been

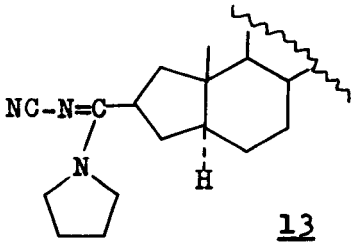
cited[62]; it can replace pyridine for this purpose. tert-Butanol may be acetylated in 15-20% yield with acetyl chloride in the absence of outside base. In the presence of urea or pyridine, the yield increases to 50-60%.

A greatly simplified preparation of cyclodiazomethane (3H-diazirine) involves treatment of methylene bis-trichloroacetamide with alkaline hypochlorite[63]. The starting bis-amide is easily prepared in one step in almost quantitative yield. 3H-Diazirine has previously been shown to undergo reactions of preparative interest[64].

An isoxazole annelation reaction has been utilized to prepare (inter alia) dl-progesterone and dl-homotestosterone[65]:



Enamines of cyclic ketones react with N_3-CN in a ring contraction; the enamine of pyrrolidine with 5-α-cholestane-3-one is converted into the A-homosteroid 13[66]:



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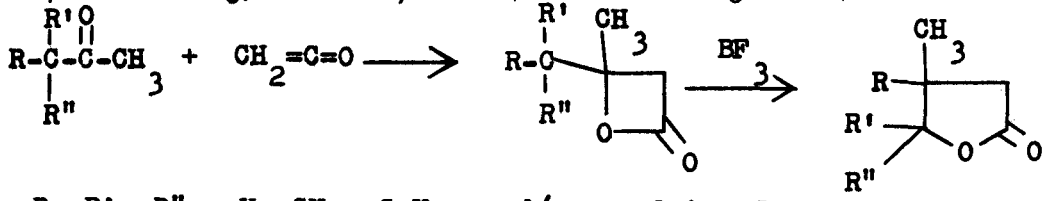
Reaction of 1-N-pyrrolidinocyclohexene with p-nitroacetophenone or ethyl p-nitrobenzoate results in reduction of the nitro group to amino and formation of 2-N-pyrrolidinocyclohex-2-en-1-one[67]. Reduction of nitrobenzene to aniline is sluggish; the enamine is converted to o-pyrrolidinophenol, not to a cyclohexenone. Literature procedures for preparation of enamines via titanium-amine complexes, $Ti(NR_2)_4$, are often hampered by the somewhat tedious methods available for preparation of the titanium reagent. An improved enamine synthesis[68] utilizes a stoichio-

metric mixture of $TiCl_4$, the secondary amine, and the aldehyde or ketone, in which the enamine is formed directly and rapidly. $TiCl_4$ is an efficient water scavenger and it also acts as a Lewis acid to polarize the carbonyl bond. This is an excellent method for preparation of highly sterically hindered enamines. The first report of C-alkylation of an aldehyde enamine[69] utilizes enamines derived from n-butyl-isobutylamine. Success in C-alkylation is due to prevention of N-alkylation by the bulk around the nitrogen.

A highly stereospecific synthesis for 1,2-trans-glycosides utilizes a reaction of sugar 1,2-orthoacetates with alcohols in the presence of $HgBr_2$[70]. 3,4,6-Triacetyl-glucose-1,2-orthoacetate reacts with cholesterol to afford a 48% yield of the pure β-linked glycoside. New and improved routes for preparation of sugar orthoacetates add to the value of the method.

The well-known preparation of β-lactones by reaction of ketene with carbonyl systems has been modified such that if the reaction is performed on a methyl ketone in the presence of BF_3, the initially formed β-lactone rearranges to a γ-lactone[71]:



R, R', R" = H; CH_3; C_2H_5; and/or cyclohexyl

2,2-Dialkoxy-2,3-dihydrofurans, preparable from a diazoketone-ketene acetal reaction, can be hydrolyzed to γ-ketoacids or esters, providing a new general method for these systems[72].

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Chapter 31. Antiradiation Agents

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Introduction - This report is for the most part based on articles selected from the 425 included in the author's bibliography[1] covering 1967. As in the two previous reports in this series no attempt has been made to cover developments in radiobiology or radiation biochemistry such as the effect of radiation on cellular kinetics[2] or the development of a mathematical model for the biological effectiveness of radiation.[3]

During the past year the world-wide effort in the areas covered by this report was maintained at the same level as in the preceding few years and there was no significant change in the relative amount of attention being given to the study of mechanisms of damage and repair on the one hand, and to the synthesis of effective antiradiation drugs on the other.

The WRAIR Program - A major advance in available knowledge about antiradiation drugs came with the removal of security restrictions from the extensive program sponsored by the Department of the Army in the Division of Medicinal Chemistry, Walter Reed Army Institute of Research. This continuing program began in 1959 and has involved the participation of about 60 laboratories operating under grants and contracts with WRAIR as well as in-house activity at WRAIR.

Most of the chemical synthesis work in the program has been published, but the radioprotective effectiveness of the drugs in bacteria, mice, and higher species was described in only qualitative terms. No decision has been reached concerning the manner in which the quantitative pharmacological data will be published by WRAIR; hopefully these will appear during 1968.

A consideration of published chemical syntheses supported by WRAIR shows that the program has been restricted to aminothiols and aminothiol derivatives having a 2 or 3 carbon atom chain between the two functional groups. In retrospect, the decision to so restrict the program seems to have been a wise one, since the aminothiols continue to be the only chemical class that protect mammalian cells by their direct presence in the irradiated cell. It is known that approximately 3000 such compounds have been studied to date in the WRAIR program.

Selected drugs have been evaluated in other areas of medicine including their use to alleviate surgical shock and arthritis, and as antagonists for nitrogen mustards. The evaluation in these latter areas is not yet complete.

Reviews - The most recent comprehensive review was that of J. B. Little,[4] who included a lengthy discussion of intracellular recovery processes.

Conventional reviews[5,6] and treatise literature[7,8,9] are also available; the last cited reference is primarily concerned with the mechanism of damage. Specialized reviews appeared covering work done at Sloan-Kettering during the period 1950-1965,[10] the influence of physiological states on radiation response,[11] space radiation biology[12] and protection.[13] An annotated bibliography covering radiobiology during the period 1898-1957 is available.[14]

Drug Evaluation - Medicinal chemists differ from the ordinary breed in that they retain an interest in the fate suffered by their hard-won compounds once they have been placed in the hands of pharmacologists for evaluation. They are, therefore, interested in drug evaluation technics. "Standard" evaluation procedures today include the use of bacteria and mice as test organisms, with some evaluation in dogs and other higher species. It is unlikely that any significant evaluation of radioprotective activity will be carried out in human volunteers.

In the case of rodent tests reports continue to appear that stress the effect on survival of balanced diets[15] and of the time of day when the animal is irradiated.[16] Even if one allows for the obvious differences between the bacteria and mouse test systems it is unfortunate that a better correlation between the two systems does not exist, even for a single class of drugs.[17] A modification of the basic concepts of radiation dosimetry to include a physical quantity other than absorbed dose was recommended to permit a better correlation with biological effects.[18]

In the case of the important aminothiol class of drug, spleen colony counts[19] and protection of cells in tissue culture[20] were used for evaluation, and the protection of the deoxyribonucleoprotein quaternary structure was also recommended as a screen for such drugs.[21]

Other new rapid screening technics included inhibition by drugs of the release of inorganic phosphate from baker's yeast under irradiation[22] and inhibition of the reaction between irradiated oleic acid and carotene.[23] It was observed that gamma-irradiated mouse tissue homogenates[24] or albumen[25] undergo a chemiluminescent reaction and the inhibition of this reaction by radioprotective drugs was used for the evaluation of these.[26] The chemiluminescent reaction was ascribed to free peroxy radicals formed in the lipids of cell membranes.[27] It is likely, therefore, that this test simply measured the radical-trapping power of the drug, which is probably not its only mode of protection.

For some time the use of plant cells as test systems has attracted investigators. MEA and other aminothiols protected plant seeds[28] and spores,[29] and a wide variety of substances, none of which are recognized antiradiation drugs, protected onion root cells.[30] One gets the impression that the replacement of bacteria and rodents by plants in the drug screen is unlikely in the forseeable future. The use of seeds for studying genetic effects of radiation is well known.

Drug Development - During this report period a great many articles de-

scribed continuing studies of the established drugs and the synthesis of new protective compounds. A critical review of the latter is not possible because quantitative pharmacological data are usually sparse or non-existent. Until such data are published we cannot safely conclude that the newer drugs are in any way superior to the older ones. The bibliography[1] referred to above should be consulted for complete coverage of drug development.

An extensive review of the pharmacological properties of important drugs appeared.[31] More recent data on aminothiol drugs included their activity as β-adrenergic inhibitors,[32] effect on sodium transport and absorption,[33] effect on oxidative phosphorylation in liver,[34] effect on amino acid content of liver and spleen,[35] effect on respiration of mice,[36] and effect on leukopoiesis.[37] When mercaptoethylamine was administered to dogs a delayed undesirable hypotension ensued, accompanied by an increase in circulating histamine.[38] This may have been the result of oxidation in vivo to the related disulfide, cystamine, which is known to cause hypotension promptly.

The fate and distribution of the well known drug serotonin was described,[39] and also cytological data relevant to its use as a protective drug.[40,41]

The as yet unexplained protective effect of dimethylsulfoxide (DMSO) on a variety of tissue cultures and animals[42] was explored in greater detail by Moos and his associates. Pre-irradiation topical application through the tail gave significant protection to mice against 760r[43] and counteracted post-irradiation saccharin avoidance.[44] Protection was also observed when mice were confined in a chamber containing an equilibrium pressure of DMSO vapor;[45] even greater protection was observed when the liquid phase was a 7% solution of Nembutal in DMSO. How this latter modification could significantly effect the dose of DMSO absorbed by the mice is not apparent. Perhaps the agile mice were able to dip their tails into the solution and absorb the barbiturate by a DMSO-promoted process. Protection by DMSO was independent of oxygen and showed synergism with aminothiol drugs. When protecting rats against 800r at 4.5 g/kg, i.p., its mechanism of action did not involve primarily the induction of hypoxia,[46] or changes in plasma enzymes.[47] DMSO protected lactate dehydrogenase, probably by formation of a metal complex.[48]

Field and his associates prepared a variety of novel disulfides and observed "good" protection in mice with 2-acetamidoethyl acetyl disulfide[49] (< 50 mg/kg), with p-cyanobenzyl 2-(n-decylamino)ethyl disulfide[50] (p-CH_3, p-CHO and p-Cl analogs were inactive) and with 3-acetamidopropyl 2-(n-decylamino)ethyl disulfide[51] (30-50 mg/kg). The 2-acetamido isomer of the last-named compound was much less protective. Thiamine tetrahydrofurfuryl disulfide (40 mg/kg, i.p., post-irradiation) protected mice against 600-700r with a dose reduction factor of 1.34.[52]

Additional examples of phosphorothioate drugs were described. $H_2NCH_2CH_2SP(O)(OH)(ONa)$ ("Cystaphos") was the most protective of 20 amino-

thiol derivatives studied[53] and its absorption in tumors was just 40% of that in other organs.[54] It is believed that in all phosphorus-containing drugs the phosphorus-containing group acted only to facilitate transport. The lithium salts of S-(3-amino-2-hydroxypropyl)phosphorothioate and S-(2-aminopropyl)phosphorothioate protected mice with dose reduction factors of 2.16 and 1.96 respectively (MEA=1.84); the purely inorganic diammonium salts of amidophosphorothioic and thiodiamidodiphosphoric acids gave dose reduction factors of 2.30 and 2.16 respectively, at molar doses 10-20 times lower than those used with MEA.[55]

A drug mixture, whose active principle is thiazolidinecarboxylic acid, gave 70% 30-day survival in rats (cysteine=8%) at 30 mg/kg against 860r.[56] Thiol groups liberated by enzymatic cleavage during the thiazolidinecarboxylic acid cycle were believed to be more protective than those derived from the drug mixture.

The possibility of using reservoir forms of potent antiradiation drugs, by analogy with similar technics in the treatment of cancer, has been explored by Friedman and associates. A correlation between the radioprotective effects of MEA derivatives and the widely-varying rates at which they liberated MEA in rat tissues was attempted.[57] The N-(2-mercaptoethyl)carbamoyl derivatives of glycine and alanine released significant levels of MEA in the stomach, spleen, and thymus of rats. Analogous derivatives of phenylalanine, methionine, aspartic acid and β-alanine released no MEA in 15 tissues examined.[58]

A dose reduction factor of 3.8 in mice was achieved by combining a variety of drugs and factors.[59] These included: MEA (200 mg/kg, 15 min. pre-irradiation), hypoxia (nitrogen atmosphere during irradiation), syngeneic bone marrow (22 hrs. post-irradiation), sodium penicillin G (50,000 I. U., 30 days) and streptomycin (50 mg/kg, 30 days).

Mechanisms of Damage and Protection- During 1967 investigations continued in all the areas summarized in last year's report, but greater emphasis was placed on fundamental radiation-induced processes in important cell components,[60-63] particularly DNA,[64,65] which would lead to mitotic failure. There is substantial evidence that DNA is the site of the significant primary lesion in the cell.

Earlier studies of the reaction of the hydrated electron with simple amino acids were extended to include di- and tripeptides,[66] in which primary amino groups were cleaved provided they were attached to a carbon atom alpha to an unsaturated function. The determination of the absolute rate constants for reactions with peptides and proteins led to the conclusion that such reactions account for only a portion of the chemical changes occurring in irradiated protein.[67]

The number of breaks in the DNA chain produced by x-rays was the same in cells and in isolated DNA, and leukemia cells were found to have a very efficient system for repairing such breaks.[68] Gamma-irradiation of

RNase brought about changes considerably more profound than disulfide or hydrophobic bond cleavage and effectively prevented refolding.[69] The considerable amount of earlier work in which electron spin resonance spectroscopy (ESR) was used to identify the specific site of damage in irradiated nucleic acids was reviewed[70] and such studies continue.[71] The attack of hydroxyl radicals on purine bases occurred at the 5 and 6 positions except in the case of adenine, which was unreactive.[72] GED at 10^{-3} molar protected DNA by binding to it and carrying out localized radical scavenging on its surface.[73] While the degradative effect of radiation on DNA continues to be studied, there is some opinion that the more serious matter is the effect of irradiation on DNA synthesis.[74]

Although ESR continues to be used widely for the identification of specific sites of damage proteins and other cell constituents, the spectrum obtained may be misleading since it can be generated by the lyophilization process used.[75] Within the limits of the experimental technics now in vogue it appears that amino acids vary considerably in their stability to radiation,[76] that the odd electron is delocalized in glycine peptides,[77] while in the case of sulfur-containing amino acids it migrates to sulfur even in the solid state with a relatively low energy of activation.[78] ESR data for irradiated proteins showed that the migration and trapping of free radicals required specific interactions and characteristic structures found in the native state.[79] The appearance of the characteristic ESR spectrum was not necessarily associated with a loss of biological properties;[80] this is in accord with the fact that the transfer of the odd electrons to thiols in frozen aqueous mixtures occurred from a limited number of sites on the macromolecules.[81]

The formation of free radical sites in irradiated living systems was detected by suing these sites to initiate copolymerization with acrylamide[82] and with vinylpyrrolidone.[83] The results of this work were in accord with ESR data.

Previous reports have described the hypothesis that copper ions are involved in the radiolytic inactivation of biological systems. Cupric ions bound to specific amino acid residues of enzymes enhanced sensitivity to hydroxyl radicals; if the ions are bound to non-essentical sites these same ions may protect the enzyme.[84] The sensitizing action of the free copper ions[85] may be avoided by catalysing their binding to plasma components with cysteine[86] and presumably with other aminothiol drugs. The protective action of AET and serotonin was markedly decreased when zinc, nickel, and cobalt salts were administered concurrently.[87]

The mixed disulfide hypothesis for the repair action of aminothiol drugs continued to draw support.[88] A variety of aminothiols exchanged readily with 4,4'-dithiobis(benzenesulfonic acid) but no simple correlation was found between the equilibrium reached and the antiradiation potency of the particular aminothiol.[89] While the presence of thiol groups in protein may be a sufficient feature for the operation of the mixed disulfide mechanism it is not a necessary feature, because amino-

thiol drugs protected enzymes that contained no sulfur.[90] The conventional radical scavenging feature of aminothiols probably functions in these and many other cases.[91,92] The protective action of disulfide drugs such as cystamine and cystine has been attributed to their conversion in vivo to the related aminothiols. Recent work on the radiation chemistry of aqueous solutions of these disulfides indicated that they function as radical acceptors and oxygen scavengers[93] in processes that involve oxidation to sulfonic acids.[94] An alternative mechanism of protection may involve a persistent change in cell permeability to potassium and magnesium ions noted in rabbits when cystamine was administered at 150 mg/kg.[95]

The protective effect of endogenous thiol groups is well known. Soviet workers continued to support the idea that aminothiols,[96] indolylalkylamines (and consequent anoxia)[96,97] and even hypothermia[98] all protect because of a marked increase of endogenous thiol concentration in sensitive organs.

The "biochemical shock" mechanism of protection, discussed last year, continued to be supported by new data[99-102] collected in experiments involving a variety of drugs.

Ever since the radioprotective effect of tissue anoxia was established years ago there has been an increasingly successful effort made to relate the mode of radioprotective drug action to this important factor. With minor exceptions[103] the protective activity of serotonin and p-aminopropiophenone is clearly the result of a significant lowering of oxygen tension.[103-106] The aminothiols may protect for the same reason, for they gave no added protection under anoxic conditions.[107] However, another study[106] showed that while serotonin lowered both oxygen tension and tissue redox potential, the aminothiols affected only the redox potential. In model experiments using thermally oxidized methyl oleate both types of drugs reduced the peroxide level by serving as peroxide decomposers,[108] while "antioxidants" of the propyl gallate type did not;[109] the latter type of activity clearly involves radical scavenging. The conclusion was reached some time ago that low oxygen tension was protective because of the consequent infrequent capture of the hydrated election,[110] or other primary cell radiolysis products. An alternative explanation ascribed the oxygen effect to accumulated endogenous substances that are simply incompletely oxidized substances.[111]

It is likely that additional data on the relation between aminothiol drugs and the oxygen effect will be accumulated in the coming year. The function of aminothiols as decomposers for perioxides in biological macromolecules might account for the high degree of structural specificity that the radioprotective aminothiols exhibit.

Cells die or are altered through damage to their DNA or to their extranuclear components, and these deaths and changes affect the general physiology of the animal. It has been suggested that protection against low-level chronic radiation by prophylactic doses of antiradiation drugs may prevent ageing in cells. There exists a possibility that knowledge

of these drugs may find significant application in gerontology.[112,113]

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Chapter 32.

Pharmaceutics, Pharmacokinetics and Biopharmaceutics

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Introduction - The availability of the drug from the dosage form depends on physical chemical factors, chemical interactions and a priori predictions and confirmations of release in biological systems.

Drug Dissolution and Diffusion - The importance of the relationship between drug absorption and the rate processes of diffusion and dissolution has been well established in recent years. W. Higuchi[1] has reviewed the literature and extracted the physical and mathematical treatments of drug transport. He considered the laws governing the dissolution rates of solids as affected by agitation, particle size, interaction with solution additives and drug release rates from matrices that do and do not obey Fick's law. The diffusion rates of pentobarbital, salicylic acid and urea across the isolated rabbit mesentery indicate diffusion of both ionic and non-ionic forms of the drugs across the membrane, with no significant change in rate due to pH[2]. Drug transport through nonpolar liquids as models of living membranes indicated that when the Hildebrand δ value of the drug molecule approached that of the lipoidal barrier, transfer of the drug molecule across and through the lipoidal barrier was faster[3]. When aqueous diffusional barriers on each side of the lipoidal membrane are included, Fick's law does not fully hold in this case of protonation of weak bases[4]. The transfer of amidopyrine and salicylic acid through an organic liquid obeys the theoretical equation proposed[5]. The formation of complexes can modify the rate of transfer of drugs through such barriers and demand modification of the diffusional equations[6]. When micron-sized emulsion droplets act as sinks for drug, equations were derived for micellar solubilization to take into account the possible effects of an electrical barrier between the micelle and the charged oil-water interface[7]. Drug release from water in oil emulsions as a function of pH into an aqueous sink[8] and with a separating membrane[9] was studied and the experimental data agreed with the physical models suggested. In order for *in vitro* dissolution studies of poorly soluble drugs to bear any relationship to *in vivo* observations, sink conditions must be maintained and this can be simulated by the presence of an organic solvent phase[10]. The kinetics of simultaneous determinations of *in vitro* drug dissolution and partitioning rates in a single system has been studied, as affected by agitation and temperature[11]. The effect of different polymorphic forms on the rate of dissolution and subsequent absorption of a drug has been shown to be of extreme importance before recent governmental committees. Suspensions containing only chloramphenicol polymorph B gave significantly higher blood levels than suspensions containing only polymorph A, regardless of particle

size[12]. The rates of reversion of the metastable to the stable forms during dissolution were correlated to the crystal growth rates for polymorphs of sulfathiazole and methylprednisolone[13]. Solvents have a distinct influence on the shape of crystals and can promote the formation of unstable polymorphic modifications[14].

Particle size has a decided effect on the rate of dissolution of drugs. Examples of the interrelation of particle size, solubility, absorption and therapeutic activity illustrate their importance in medicinals for oral, topical or parenteral administration[15]. The angle of repose and sliding angles of sodium borate and boric acid powders were calculated and it was found that the cohesive force between the particles is negligibly small for particle diameters larger than the critical size while it significantly influences the angle at smaller diameters[16]. The apparent specific volume of samples in the loosest packing is nearly constant for sizes above the critical diameter, but it increases gradually with decreasing particle size below the critical diameter[17]. The angles of repose were apparently lowered by the addition of the lubricants[18]. The Coulter counter[19] was used to study the particle size distribution of various samples of griseofulvin. The considerable differences which would affect specific surface area and thus blood levels demanded stricter official specifications[20].

Dissolution test procedures which have not been calibrated on the basis of in vivo measurements are unlikely to reflect the gastro-intestinal absorption and may in fact yield misleading data[21]. Intrinsic dissolution rates varied considerably among tablets for aspirin[22], sodium salicylate[23] and phenindione[24], depending on the crystalline form, compression and tablet technology. A new dissolution apparatus permits 20 dissolution tests simultaneously and the results agree well with standard procedures[25].

Drug Interactions - The first order rates of deaggregation abbetted by wetting and dispersing agents of a suspension of a substituted benzoic acid and its sodium salt correlate with the rates of solution[26]. The theoretical simultaneous release of a mixture of two non-interacting drugs dispersed in an inert plastic matrix was confirmed by studies on a salicylic acid-benzoic acid plastic mixture[27]. The validity of the model for the interacting system, benzocaine-caffeine, was also verified[28]. Surprisingly, the release of salicylic acid from polyethylene matrices exhibited no rate increase in the presence of increasing concentrations of dibasic potassium phosphate even though the solubility increased as much as 45 fold. The presence of a surfactant, however, produced a dramatic rate increase. The result indicated that matrix permeability and rates of permeation by the solvent can restrict drug release rates as a function of pore size distribution of the matrix and wetting properties of the solvent defined by surface tension and contact angle[29]. The release of benzoic acid and salicylic acid into aqueous media from wax matrices are best analyzed from a diffusion controlled model and square root of time release profiles[30]. Over one range of composition the rate of release of a hydrogenated castor oil-propylene glycol monostearate-sulfanilamide is diffusion controlled, with high tortuosity values. At higher sulfanilamide concentrations in the tablet, the release is not diffusion controlled[31]. Dissolution rates of

hexestrol, dienestrol and griseofulvin increased significantly in the presence of lysolecithin[32] as they did for testosterone in aqueous solutions of polysorbates[33]. In general, increasing concentrations of surface-active agents increase the dissolution rates of drugs[34]. Non-ionic surface active agents interact with preservative, decrease their antimicrobial activity, and affect the over-all absorption of the medicaments[35]. Polysorbate 20 and a polyelectrolyte dispersant increased the release rates of solids in microcapsules[36]. Chemical, physical and morphological reasons for the permeation of various chemical structures through plastic materials were postulated[37]. A series of structurally related aromatic compounds were studied for their ability to permeate through a polyethylene film. Benzyl alcohol and benzoic acid had the lowest permeability due to intermolecular hydrogen bonding[38].

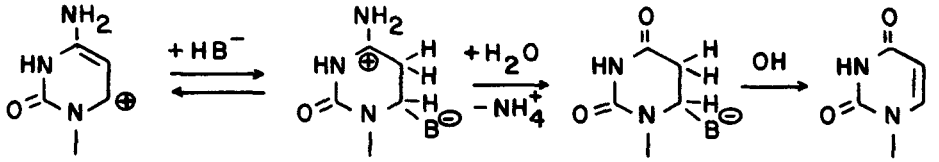
Drug Stability - The theoretical bases for formulation of stable drugs depend upon knowledge of the kinetics of the thermal solvolysis of the drug in solution as a function of pH, concentration and excipients. This section considers recent fundamental contributions on the kinetics and mechanisms of drug stability. A series of review papers on the kinetics and stability of drugs has recently been published[39-41]. A complete and critical review by Garrett[42] of the available literature up to 1966 on kinetics and mechanisms in the stability of drugs is now available. The hydrolysis of undissociated aspirin in buffered solutions of the non-ionic surfactants, cetomacrogel[43] and polysorbate 80[44] is inhibited due to the partition of, and thus the unavailability in true solution of, aspirin[43]. A specific hydrogen ion catalyzed hydrolysis of the undissociated aspirin partitioned into the micelles is observed but is of a smaller magnitude than its counterpart in true solution. Dissociated aspirin is not partitioned and shows no overall inhibition of solvolytic rate in the presence of the surfactant[43]. Cyclodextrin enhances the hydrolysis of aspirin at elevated pH values but inhibits the hydrolyses of ethyl aminobenzoates and atropine[45]. The former is ascribed to the steric juxtaposition of ionized hydroxyl groups to bound aspirin whereas the latter is ascribed to inclusion phenomena where the solution availability of the substrates is reduced[45]. It was also shown that the specific hydroxyl ion catalyzed hydrolysis of benzocaine and homatropine in the presence of nonionic surface-active agents is unaffected at less than the critical micelle concentration[46]. However, at a concentration in excess, the stability is enhanced due to micellar interaction or partition. Fersht and Kirby[47] have demonstrated that the pH independent region for the solvolysis of aspirin is due to the fact that the ionized carboxyl group acts as a general base in its attack on water to form the hydroxyl ion that attacks the ester, rather than the previously accepted mechanism of intramolecular nucleophilic attack on ester carboxyl carbon that results in a mixed anhydride. This occurs when aspirin is substituted with highly electron withdrawing groups[48]. General base catalysis by acetate and phosphate was also demonstrated. The attack of nucleophiles on the ester group is promoted by intramolecular general acid catalysis effected by the undissociated carboxylic acid group[49].

Schwartz[50], as part of a continuing and detailed series on model catalysts which simulate penicillinase has studied the mechanism of hydrolysis of penicillin catalyzed by catachol and their pH dependencies. The degra-

dation of 6-aminopenicillanic acid by solvolysis of the β-lactam is first order below pH 6.6 and tends toward second order with respect to substrate above this value[51]. The pH of minimum solvolysis is 8.0. A concomitant reaction is dimerization through the nucleophilic attack of the amino group of one molecule to the β-lactam of another to form penicillin. The kinetics of a series of 3,4-dialkylsydnones were studied where the presence of an α-hydrogen ion on the alkyl substituents tended to stabilize against hydrogen ion and water attack, probably by decreasing the concentration of a reactive intermediate with equilibrated tautomeric forms[52]. The rate-pH profile and Arrhenius' parameters have been determined for echothiophate iodide[53]. The kinetics show a pH independent solvolysis with

$$(CH_3)_3N^+ - CH_2 - CH_2 - S - O(O)(O - C_2H_5)_2I^-$$

the loss of one mole of ethanol and a specific hydroxyl ion attack on the S-P bond to yield (2-mercaptoethyl)-trimethylammonium iodide. Notari[54] has stated that the primary mechanism of hydrolytic deamination of cytosine arabinoside appears to result from the attack of the nucleophilic monoanion of a polybasic acid (bisulfite, dihydrophosphate, etc.) with a readily dissociable proton on the activated C-6 position of the protonated nucleoside, I, with subsequent saturation at the C-5 position by the labile proton, II. Subsequent nucleophilic displacement by H_2O occurs at C-4 with a loss of NH_3. The products II and III are isolable. Further alkaline treatment can regenerate the uracil arabinoside



The hydrolytic rates of various N-substituted 6-amino-thiouracils to the corresponding 6-aminouracils were compared in strongly acidic and alkaline solutions and were enhanced over the unsubstituted compound[55]. At high acid concentrations, the pH independent attack of water on the protonated species becomes rate-determining. The rates of formation of hydroxymethylfurfural from fructose and sucrose[56], of furfural from ribose[57], and of the now identified 5-methyl-3(2H)-furanone from 2-deoxy-D-ribose[58] have been characterized as a function of temperature and acid concentration. The kinetics of degradation of these products have been characterized as a function of temperature and alkali concentration[56-58]. Isoniazid shows an anerobic hydrogen ion catalyzed solvolysis with significant buffer effects to isonicotinic acid that can be assigned to hydrogen ion attack on the monocation or water attack on the dication[59]. Certain sulfa drugs such as 2-sulfanylamido-4,5-dimethyloxazole and 3-sulfanylamido-2-phenyl-pyrazole are highly susceptible to oxidative degradation through sulfanylurea to sulfanylamide[60]. The interesting observation of an unusual acid-catalyzed solvolysis of an anhydride was observed by Nestler and Seydel in the case of the isonicotinic acid anhydrides[61]. The kinetics of solvolysis of 2-ethylpyridine-4-carboxythioamide have been described. It undergoes solvent and specific acid catalyzed solvolysis to the corresponding carboxylamide and carboxyl, and in alkali forms both stable carboxylic acid

and thiocarboxylic acids[62]. The reaction of the food preservative, dehydroacetic acid with amino compounds under in vitro physiological conditions has been studied in ethanolic solutions. Schiff base formation as a function of pH is maximal ca. pH 7 and is consistent with the classical mechanism of attack of undissociated amines on the protonated carbonyl carbon[63]. The use of carbonate esters as "prodrugs" which possess desirable pharmaceutical properties and can release pharmacologically active compounds on hydrolysis in vivo has been suggested[64] and thus the study of their enzymatic hydrolysis of α-chymotrypsin was conducted[65]. The rates were proportional to enzyme and substrate concentration where the former could be saturated in accordance with typical Michaelis-Menten kinetics. A sigmoid pH-rate curve is obtained characteristic of an enzyme function of pK_{a_2}. There is a very rapid production of alcohol, P_1, and a subsequent slower rate when enzyme concentration, E, is deficient. This can be explained[65] on the premise that the regeneration of enzyme, E, along with the acyl moiety, P_2 from a partially dissociated enzyme complex ES' becomes rate determining

$$E + S \rightleftharpoons ES \longrightarrow P_1 + ES' \longrightarrow P_2 + E$$

An apparatus for solution kinetics to minimize lag time for thermal equilibration and oxygen contamination has been described[66]. The maintenance of constant spectrophotometric absorbance of an acid-base indicator while monitoring added acid-base reactant has been proposed and evaluated to maintain constant pH in the hydrolysis of esters[67].

Pharmacokinetics and Biopharmaceutics - The quantification of the distributions of acute doses of a drug and its metabolites in the multicompartmental complex organism and its rates of metabolism and excretion as functions of dose are necessary prerequisites for the determination of its biological availability from dosage forms and depends on perturbations of the time-course of observed distributive patterns. The limitations of the simplifying hypothesis of a rapidly equilibrating total volume of distribution to validly estimate the first order rate constants for loss of drug from the blood and for accumulation in the urine have recently been appreciated [68,69]. Examples of the invalidity of this hypothesis are referenced in a discussion of the limiting conditions for observing simple exponential loss of drug from the blood plasma[70]. This simplifying postulate is still used in computer programs for the simple model of first order appearance and first order removal of drug from the blood[71,72] which permit determination of both rate constants even when they are of similar magnitude[72]. Methods have been proposed[73] for estimating first order rate constants of absorption, metabolite formation and overall loss of drug from the body when the time course for urinary excretion of a metabolite is known and the rate constant for urinary excretion of the metabolite is available.

Wagner[74] has claimed that evaluation of the time course of urinary excretion of metabolites on the assumption of rapid clearance may lead to an erroneous conclusion of saturation of metabolic pathways. He also stated that the apparent linearity of a cumulative urinary excretion curve and/or the curvature of semilogarithmic plots of drug blood levels or of amounts of drug-not-excreted against time are insufficient evidences to conclude zero order steps in metabolite production or zero order absorption

of a drug. He used data in the literature on aspirin pharmacokinetics as his examples. Levy[75] has claimed that additional and strong evidence is available for the pharmacokinetic model for salicylate elimination in man which assumes capacity-limited or zero order formation of salicyluric acid at higher doses.

If the time-course of drug levels in the accessible compartments of the body is correlated with the time-course of pharmacological activity, insight can be gained into the properties of the compartment in which reside the receptor sites[70,76]. Methatrimeprazine levels in the brain were correlated with the onset and duration of pharmacologic effects and implicated direct action and distribution of the parent compound . The overturn time for goldfish and its duration were used as a pharmacologic endpoint for the action of ethanol and pentobarbital and is a function of body drug levels and rate of drug elimination[78].

Urinary excretion studies[79] have shown that riboflavin-5'-phosphate and riboflavin are incompletely absorbed in the upper region of the gastrointestinal tract and the absorption sites appear to be saturable since the percent recovery decreases with increasing oral dose. Enterohepatic cycling is also indicated. Probenecid inhibits the specialized transport process responsible for the intestinal absorption of riboflavin in man and apparently inhibits the active renal tubular secretory process, thereby increasing the apparent blood level half-life[80]. Further work has been carried out on the pharmacokinetic model for nalidixic acid in man[81]. The blood levels on repeated dosing could be predicted from the pharmacokinetic model obtained from the study of an acute dosage and demonstrated no saturation or induction effects. The pharmacokinetics and metabolism of 5-methylpyrazole-3-carboxylic acid has been studied in the rat, dog and human and no variations in the pharmacokinetic parameters were observed on chronic dosing. The drug is completely absorbed and rapidly excreted with an acidic conjugate as a partial metabolite in the rat and man[82]. The apparent half-life of diazoxide in the blood of man was determined to be 28± 8.3 hrs.[83]. Thiothixene is well absorbed, rapidly distributed and metabolized to products which are biliary excreted[84]. The absorption of ^{14}C-labelled teroxalene HCl in various species showed a linear relation to oral dose and apparent first order elimination although large residuals were maintained in adipose tissue[85]. The absorption, excretion and metabolism of dimethylsulfoxide has been studied in man and unchanged drug and the metabolite dimethylsulfone are excreted in the urine[86]. Salicylamide is eliminated as the glucuronide and the sulfate, the former decreasing with increasing dose and the latter increasing and imply saturation processes[87]. Probenecid inhibits efflux of 5-hydroxyindoleacetic acid from rat brain to plasma in steady-state kinetic studies[88]. Rifampicin, a new rifamycin, has been pharmacokinetically studied as to absorption, diffusion and elimination in humans[89]. The pharmacokinetics and metabolism of chlormezanone has been studied in man and laboratory animals[90]. p-Methoxycinnamate is rapidly absorbed in rabbits and is rapidly metabolized to p-methoxybenzoate[91]. Blood level half-lives are greater on oral than on intraveneous administration. The maximum concentration in the blood is proportional to the dose. The first order loss of intravenously administered bishydroxycoumarin levels in plasma has been compared for the rat,

guinea pig, dog and rhesus monkey[92]. Only in the monkey was it definitive that the half-life increased with increasing dose. Methyridine is rapidly absorbed and distributed in sheep, calves and cows on various routes of administration[93]. The major metabolite is pyrid-2-ylacetic acid which is also excreted as a glycine conjugate. Increase of drug dosage to compensate for enzyme induction may result in marked toxicities[94]. Different administrative routes of cholinesterase inhibitors vary their toxicities and it is suggested that the availability for metabolism in the liver is the major factor[95]. The total urinary excretion of amphetamine is pH dependent but there is a definitive difference in the relative excretion rates of the isomers[96]. The pharmacokinetic models for cyproterone acetate were similar in men and baboons and significant gastrointestinal resorption was observed[97].

On the hypothesis that modifications of the rate of drug absorption may control or reduce nausea and/or emesis associated with the difficultly soluble nitrofurantoin administration, the crystal size was controlled and availability in rat, dog and man, and emesis in the dog were shown to increase as a function of the available surface area of fine crystals[98]. Griseofulvin absorption in man correlated with its dissolution rates in various formulations in simulated intestinal fluids[99]. The time-courses of dextroamphetamine ^{14}C-sulfate[100] in dogs and humans and amobarbital-^{14}C in humans[101] were monitored in plasma and urine to compare the availability from sustained and nonsustained-release dosage forms. Biological availability from various formulations for rectal absorption[102,103] were determined from blood levels. Plasma levels of phenmetrazine were evaluated from different formulations[104]. Relative absorption rates of penta erythritol tetranitrate from ligated sections of the gastrointestinal tract were attributed to its degradation into lower nitrates[105]. Absorption of quinine hydrochloride from enteric coated tablets has been monitored by plasma and urinary levels with time and its absorption is decreased in the distal parts of the intestine[106]. Urinary excretion data was used to study the release rate in vivo of antipyretic and analgesic drugs from commercial sugar-coated tablets[107]. Polysorbate 80 enhances the absorption of secobarbital in the goldfish by increasing the permeability of the biological membrane[108]. The efficiency of tetracycline absorption in different subject panels has been shown to be similar based on the sequential first order model of absorption and excretion and premise that the area under the blood level-time curve is related to the relative absorption efficiency[109]. Similar procedures have been used to compare the enteral absorption of sulfanilamides[110]. Levy[111] has demonstrated significant differences in the pharmacokinetic constants for benzyl penicillin in humans when ambulatory or during bed rest which may be attribtuted to variations in apparent volumes of distribution that affect apparent metabolic rates. Significant differences in dissolution rates may affect salicylate availability in individuals with high absorption rates but not those that are slow absorbers[21].

Amphetamines blocked in the para position with a chlorine disappeared from the brain at slower rates which could be attributed to the blocking of the para hydroxylation route[112]. Four urinary metabolites of N-(o-aminophenyl) N- dimethylaminopropyl) anthranilate have been identified in the

dog and human[113]. The kinetics of excretion of the drug and its metabolites have indicated that absorption and metabolism take place rapidly and that the biliary route is the major one in excretion. Equilibrium dialysis studies of the protein binding of cortisol show that the chemical degradation in the finite time of the study give erroneous estimates of the binding if not accounted for[114]. Biopharmaceutical considerations in subcutaneous and intramuscular drug administration have been reviewed recently[115] as has the mechanisms of drug absorption and excretion[116].

There has been a recent awareness that metabolic and distributive patterns vary among individuals for the same drugs[117,70] and that in the future the metabolic and pharmacokinetic profiles of individuals will be mapped before prescribing drugs and dosage regimens.

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Chapter 33. Physicochemical Parameters in Drug Design

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Introduction - Interest in quantitative correlation of chemical structure and biological activity is developing at a much more rapid pace than one might deduce from the number of papers which have appeared since our last review.[1] From informal talks it is apparent that a variety of laboratories have formulated computer programs and are actively pursuing such studies. The two general approaches that are receiving attention are the empirical Free-Wilson[2] and Kopecký-Boček[5] methods and the semi-empirical method in which changes in biological activity of members of a set of congeners are correlated with known physicochemical constants.[1] We are unquestionably entering a new era where the role of the computer in drug design is becoming indispensible. As pharmacological testing becomes more precisely defined in numerical values, the drug designer faces the sharper challenge of explaining quantitative differences in physical chemical terms. Sorting out roles for the large number of variables can only be accomplished through computerized regression analysis.

De Novo Constants - Purcell and his colleagues have continued their development of the Free-Wilson empirical method in which the biological activity is expressed as a function of the activity contributions associated with segments of the molecule (substitutent groups and the parent portion). In one study[3] they have obtained *de novo* substituent constants for the contribution of hydrogen, methoxy, and various alkyl groups to the hypoglycemic activity of piperidinesulfamylsemicarbazides. From the observed biological activity of 12 derivatives they have predicted activity for 12 other derivatives. In a second study they have derived substituent constants for group contributions to the antitumor activity of acetylenic carbamates.[4] Boček and Kopecký have reported[5] an example of the use of *de novo* constants for the correlation of toxicities to mice of ortho-disubstituted benzenes.

Semi-Empirical Method - More effort is being directed toward using known physicochemical parameters to rationalize the change in biological activity resulting from the modification of a parent molecule. In this approach, attempts are being made to relate ΔBR (change in biological response) to changes in hydrophobic, electronic, and steric effects of substituents. The very great importance of hydrophobic interactions in biochemical systems is receiving increased attention.[6]

Partition Coefficients - In using an aqueous phase and a simple organic solvent to serve as a reference system for approximating hydrophobic interactions in biochemical systems, Collander[46] has pointed out that we are assuming: $\log P_1 = a \log P_2 + b$. In this relationship P_1 is the partition coefficient in one system (fatty and aqueous biophases) and P_2 is the partition coefficient in a second system (e.g., octanol-water). While Collander did provide some evidence for this linear relationship, much

better evidence is available from the earlier work of H. W. Smith. Equation 1, based on Smith's results, shows[7] a good linear relationship between the partition coefficients of a variety of acids in the two systems $CHCl_3$-water and xylene-water. In eq 1, n is the number of compounds used in the regression and r is the correlation coefficient. Other good examples validating Collander's relation come from the work of Kakemi, Arita, Hori and Konishi.[8] These workers also pointed out that for barbiturates the value of P is independent of the concentration of barbiturate partitioned. This seems to be generally true of more or less neutral molecules.[27] Of more interest is the type of correlation possible between a simple solvent system and biopolymers. That log P or π from the octanol-water system is a good parameter for such correlations can be seen from the following equations. In eq 2 and 3, K are binding constants of precise definition while eq 4 and 5 are not so sharply defined. In eq 5, B refers to the % bound and F to the % free. The work of Bird and Marshall;[10] embodied in eq 5, is most significant because of the large number of compounds studied and the great variation in their structure. The binding of sulfanilamides to plasma protein has also been quantitatively correlated using substituent constants.[11] Kakemi, Arita, Hori and Konishi have shown that the absorption of barbiturates by the rat stomach is linearly related to their partition coefficients.[12] That electronic effects of substituents can outweigh lipophilic effects in certain instances is apparent from a study of the uptake of S-benzoylthiamines by human erythrocytes.[13] In this study a good correlation was found between σ and the adsorption of a given derivative. Actually, in this case σ may correlate the degree of ionization which affects the partition coefficient.

$$\log P_{CHCl_3} = 1.064 \log P_{xylene} + 0.393 \qquad n = 30 \quad r = 0.979 \quad (1)$$

Binding of ROH by Ribonuclease[7]

$$\log K = 0.504 \log P - 1.560 \qquad n = 4 \quad r = 0.999 \quad (2)$$

Binding $RCOO^-$ by Serum Albumin[7]

$$\log K = 0.594\pi - 6.514 \qquad n = 5 \quad r = 0.966 \quad (3)$$

Binding Barbiturates by Rabbit Brain[9]

$$\log \% \text{ bound} = 0.526 \log P + 0.467 \qquad n = 4 \quad r = 0.992 \quad (4)$$

Binding of Penicillins by Serum[10]

$$\log (B/F) = 0.488\pi - 0.628 \qquad n = 79 \quad r = 0.924 \quad (5)$$

Enzyme Studies - Log P, along with the Hammett σ constant, has found use in the correlation of enzymic reactions.[1] The study of Blomquist[14] on the electronic effects of substituents in alcohol dehydrogenase reactions illustrates the point. Blomquist noted that both electronic and hydrophobic effects appear to be involved in the enzymic reduction of benzaldehydes. We have placed his results in mathematical context in eq 6 and 7 where K =

$$\log K = 0.886\sigma + 2.109 \qquad n = 5 \quad r = 0.917 \quad (6)$$

$$\log K = 0.444\pi + 0.909\sigma + 1.986 \qquad n = 5 \quad r = 0.999 \quad (7)$$

[$1/\phi_2$ derivative/$1/\phi_2$ benzaldehyde)100]. Equation 7 is a highly significant improvement over eq 6. An F test indicates the π term to be significant at $\geq$.995. The π values are from the benzoic acid system.[15] The coefficients in eq 7 are similar to those found by McMahon for the enzymic reduction of aromatic ketones to alcohols.[1] Another enzymic example is embodied in eq 8, formulated from the results of Bender *et al.*[16] for the cycloamylose-catalyzed hydrolysis of phenyl acetates. The five data points are for the meta isomers only. Equation 8 is not highly significant statistically because of the few points used in the regression; how-

$$\log K = 0.506\pi + 1.202\sigma - 1.432 \qquad n = 5 \quad r = 0.913 \quad (8)$$

ever, it does point to new directions for research. Wildnauer and Canady have used a variety of parameters to correlate enzyme-inhibitor complexes of α-chymotrypsin.[45] A considerably more complex structure-activity relationship, in terms of π and σ, was found for the inhibition of malate dehydrogenase by phenols.[17]

Nonspecific Inhibition - The use of log P in the correlation of the structure-activity relationship in a wide variety of narcotics[9] has shown a linear relation between log 1/C and log P for 16 different systems with slopes near 1. The similar equations point to a common mechanism of action, possibly the inhibition of electron transport in oxidative metabolism. The use of log P for relating the effect of different sets of congeners acting on different biological systems is indicated in eq 9 and 10.

50% Inhibition *Arbacia* Egg Cell Division by Barbiturates[9]

$$\log 1/C = 0.801 \log P + 1.076 \qquad n = 19 \quad r = 0.960 \quad (9)$$

Inhibition *Avena* Cell Elongation by Phenoxyacetic Acids[18]

$$\log 1/C = 0.778 \log P + 1.971 \qquad n = 22 \quad r = 0.928 \quad (10)$$

The slopes of the above equations are the same, indicating the same dependence of inhibition on the hydrophobic character of the drug. The intercepts are different, indicating greater sensitivity of the *Avena* test. These are arbitrary standards and it would be interesting to place both on the same basis to compare the intrinsic activity of the two classes of inhibitors. An equation similar to 10 was also found for the phenylacetic acids.[18] While both sets of acids are toxic at high concentrations, at much lower concentrations they promote cell elongation.

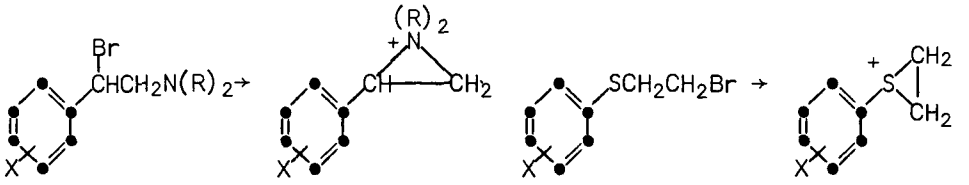
An interesting example illustrating how conformational changes in an enzyme can be quantitatively correlated using π constants comes from the work of Ichikawa and Yamano.[19] Equation 11 correlates the molar concentration of phenol causing 50% conversion of cytochrome P450 to P420.

$$\log 1/C = 0.631\pi + 1.194 \qquad n = 11 \quad r = 0.986 \quad (11)$$

The study was made using liver microsomes and 14 different phenols. Equation 11 differs from the one formulated by Ichikawa and Yamano in that the three most lipophilic phenols (2,4,6,-triCl; 2,3,4,6-tetra-Cl; penta-Cl)

were omitted since a plot of the data showed a departure from linearity at $\pi \cong 2.3$. Using a π^2 term, all points are well fit. An equation similar to 11 was also found for aniline derivatives.[19]

Alkylating Agents - Hansch and Lien[20] have shown that adrenergic blocking by β-halophenylethylamines (I) studied by Graham can be correlated by two types of equations.



I II III IV

Equation 12 correlates the effect of ring substituents (X) in compounds of type I and eq 13 correlates the effects of changes in R. In eq 12, where

$$\log 1/C = 1.221\pi - 1.587\sigma + 7.888 \qquad n = 22 \quad r = 0.918 \quad (12)$$

$$\log 1/C = 1.113E_s^c + 3.566\sigma^* - 4.432n_H + 11.911 \qquad n = 10 \quad r = 0.986 \quad (13)$$

the haloamines were studied *vs.* adrenaline in rats, the hydrophobic character of the substituent was most important. In eq 13, where the benzene ring was held constant and variations in the R groups attached to the nitrogen of the side chain were made, hydrophobic binding did not appear to play a significant role. In eq 13, n_H represents the number of hydrogens attached to the protonated nitrogen and E_s^c is Hancock's corrected steric parameter. E_s^c often appears to give better results[7] than E_s. The rationale for using n_H was that eq 14 could be used to correlate the base strengths of primary, secondary, and tertiary amines.[20]

$$pK_a = 3.140\sigma^* + 1.816n_H + 7.817 \qquad n = 92 \quad r = 0.985 \quad (14)$$

Ishida[21,22] and coworkers have studied the ovicidal activity of a set of congeners of bromoethylthiobenzenes (III). Equation 15 was derived to rationalize the toxicities. They postulated that the onium compound (IV)

$$\log 1/C = -2.24\pi^2 + 1.74\pi - 1.44\sigma + 4.34 \qquad n = 8 \quad r = 0.967 \quad (15)$$

was an important intermediate and that this intermediate might then act biochemically as an alkylating agent. In an *in vitro* study of the alkylation of 4-(p-nitrobenzyl)-pyridine with compounds of type III, they found a rho constant of -1.85 to -2.04. Rho for hydrolysis was similar, -1.71 to -1.98. These negative values of rho are reasonably close to -1.44 of eq 15 considering the different "solvent" environments. The value of rho of eq 15 is close to -1.59 of eq 12. The haloalkylamines are also assumed to act as alkylating agents via II. While one would not ex-

pect the same value for rho in these two systems, similar values would be expected.

Hill Reaction - Equation 16 correlates[23] the inhibiting action of N,N-dialkylphenylureas on the Hill reaction. Equation 16 is similar to that

$$pI_{50} = 1.78\pi - 2.44\sigma + 4.59 \qquad n = 8 \quad r = 0.87 \quad (16)$$

found for other Hill reaction inhibitors.[1]

Nonlinear Dependence of Activity on Log P - Evidence[24,25] continues to mount that while the dependence of biological activity on log P is not linear, over large ranges of P it can be treated in a rational manner using the expression

$$\log BR \equiv \log 1/C = -k(\log P)^2 + k'\log P + k'' \quad (17)$$

When electronic and steric effects in a set of congeners can be neglected, eq 17 often yields good correlations. For 16 different sets of hypnotics tested in a variety of ways on mice, rats, rabbits, and guinea pigs, an average value of log P_o of 2 with a range of 1.5-2.7 was found. While log P_o says nothing about the intrinsic activity of a set of drugs, it does set a limit to the maximum activity one can expect to reach in a given set operating by a fixed mechanism by simply increasing the lipophilic character of the parent drug. It has been postulated[24] that there should be an ideal lipophilic character for a set of drugs so that the members having this value would be least restricted in their random walk through biological tissue to the sites of action. Once the drug molecule reaches the site of action, there is a last partitioning step onto a receptor site which may be highly important in determining BR and highly dependent on log P. To bring this out, eq 17 can be written[24] as:

$$\log 1/C = -k_1(\log P)^2 + k_2\log P + k_3\log P + k_4 \quad (18)$$

In eq 18, $k_3\log P$ comes from factoring the $k'\log P$ term of eq 17 to separate the dependence of log 1/C on the last partitioning step. If we assume that the hypnotics act by a rather nonspecific mechanism and the last partitioning step is little different from the many others, the empirically found log P_o should be the same as the ideal value, log P_i, representing maximum freedom in the random walk process. There is some evidence that log P_i may be about 2. The fact that benzeneboronic acids penetrating[24] into the mouse brain have log P_o of 2.3 (here the analytical tool is not biological response, but chemical analysis for boron) supports this view. A variety of other important, more or less *neutral*, CNS acting drugs[24] also have log P values near 2. The above concept removes part of the mystery of the so-called "blood-brain barrier." When active transport is not involved, the farther one is from log P of 2, the lower the possibility a neutral drug has of penetrating the brain in a fixed time interval. When the $k_3\log P$ term in eq 18 becomes important, or when one has a situation approximating an equilibrium condition of the type envisaged by

Ferguson,[26] then log P_o will be different from log P_i. For example, log P_o for thiobarbiturates is found[24] to be about 3. For Gram-negative bacteria in *in vitro* tests, log P_o is about 4, and for Gram-positive bacteria it is found[25] to be about 6. Once one has determined log P_o for a given system by the testing of a few derivatives, considerable design work in the formulation of new derivatives for testing can be done by taking advantage of the additive-constitutive nature of π and log P.[27,28]

Nonlinear Dependence of Activity on Electronic Factors - Just as departure from linearity between log 1/C and log P occurs when large changes are made in log P, so departure from linearity between log 1/C and electronic changes results when large changes are made.[11,17,29,30] Two approaches have been applied to take account of this fact mathematically; Fujita[11,29] has suggested the approach formulated in eq 19 for expressing activity in

$$\log 1/C + \log(K_a + [H^+])/[H^+] = k\sigma + k'\pi + k'' \qquad (19)$$

terms of the unionized form of the drug. The left side of eq 19 can be replaced with log 1/C + log(K_a + $[H^+]/K_a$ for the ionized form of the drug. Equations of the type of 19 have been shown to give good correlations with biologically-active phenols[29] and sulfa drugs.[11] However, as Fujita has pointed out,[29] considerable care must be exercised in interpreting such correlations. Since there is a direct relationship between σ and K_a, one in effect "builds in" correlation in this type of expression. Equation 19 does have the advantage that changes in the external pH are included, $[H^+]$. The second approach to systems nonlinear in σ is to include a σ^2 term.[17,30]

Higher Order Approximations - In the early attempts[1] to correlate structure with activity mathematically, simple linear combinations of physicochemical parameters were usually considered. It has become evident that the addition of interaction terms to such equations can in some instances yield sharper correlations.[5,9,18,31] Singer and Purcell[31] have discussed this problem and compared the models of Free and Wilson, Kopecký and Boček and Hansch and his colleagues. They point out that in view of the many instances where BR is not linearly[24,25] related to log P and where BR is also not linearly related to electronic effects[11,17,29,30] that the model of Free and Wilson will not hold, but that the Kopecký-Boček model[32] should apply.

Polarizability and Activity - Hersch,[33] in continuing his study of the relation ln(MBC) = ln C_s - KR_oI, has investigated the interaction of local anesthetics with lecithin monolayers. In the Hersch equation, MBC is the minimum concentration of drug blocking nerve excitability, C_s is the minimum blocking concentration of molecules at the surface, R_o is the mole refraction, I is the ionization potential, and K is a function of interaction distances which is assumed as a first approximation to be constant. Hersch has shown that a good linear relation exists between log($\Delta\pi$/MBC) and R_oI, where $\Delta\pi$ represents change in surface pressure. Cammarata[34] has analyzed the attempts to correlate structure with activity in chloramphenicol analogs. He has formulated eq 20 which gives a very high corre-

lation with the single parameter P_E, molar electronic polarizability. The

$$k_I = 2.76P_E - 6.55 \qquad n = 9 \quad r = 0.991 \quad (20)$$

inhibitory constant, k_I, is from the work of Garrett. The results of Hersch and Cammarata indicate the importance of more exploratory work with P_E. This parameter might be particularly useful in separating charge transfer complexing ability of various functions from simple hydrophobic interactions.

Newer Semi-Empirical Approaches - Fuller, Marsh and Mills[47] have derived eq 21 which correlates inhibition of monoamine oxidase by N-(Phenoxyethyl)-cyclopropylamines. In eq 21, γ is an arbitrarily defined steric constant. Equation 21 was used to predict the activity of two new de-

$$pI_{50} = 0.865\gamma + 0.209\pi + 1.547\sigma + 5.928 \qquad n = 16 \quad r = 0.90 \quad (21)$$

rivatives (4-N=N-C_6H_5; 4-NH_2). Both derivatives were correctly predicted. One was found to be more active than any previously tested. The other had the expected low activity. Equation 21 was based on rat liver MAO. A similar equation was formulated for inhibitors of the human enzyme. Turner and Battershell derived eq 22 which correlates the fungicidal activity of tetrachloroisophthalonitrile and its analogs in the foliage protectant

$$\log C = -0.053(\log t_{1/2})^2 + 0.413 \log t_{1/2} + 0.513 \log VP + 1.021$$

$$n = 9 \quad r = 0.994 \quad (22)$$

test with early blight.[48] In eq 22, $t_{1/2}$ is the half reaction time of the fungicide with 4-nitrothiophenoxide and VP represents vapor pressure determined by GLC method. Since the fungicides were quite complex in structure, Turner and Battershell used the reaction parameter $t_{1/2}$. This parameter would of course contain both electronic and steric components. The thiophenoxide ion was used to represent a suspected enzymatic, -SH, in the fungus. This appears to be an extremely useful general concept to employ when one is dealing with molecules too complex for M.O. calculated parameters or σ constants. McFarland *et al.*[49] have derived eq 23 correlating structure and activity for pyrantel analogs. The addition

$$\log(1/MED) = -2.30\pi^2 + 2.83\pi - 0.320\sigma + 0.824\mu^2 + 0.988$$

$$n = 7 \quad r = 0.992 \quad (23)$$

of the dipole moment term (μ) results in considerable improvement in correlation. Other studies on dipole moments[41-44] bring out the interest in the use of this constant for quantitative correlations. Purcell has extended his studies and shown the utility of eq 24 in the correlation of cholinesterase inhibition by 3-(N,N-diethylcarbamoyl)piperidinoalkanes.[43]

$$pI_{50} = \sum_{1}^{n}\left(\frac{1}{2.8}\right)^{n-1} A + Bn + C \qquad (24)$$

In eq 24, $pI_{50} = -\log I_{50}$ where I is the molarity causing 50% inhibition, n is the number of carbon atoms in an alkyl chain, A is an electronic factor, B a hydrophobic parameter, and C is the contribution to activity of the parent portion of the molecule. Amoore[51] continues to make progress in quantitatively correlating the shape of organic compounds with their odor. The similarity of molecules is compared by scanning their molecular silhouettes with a computerized pattern recognition machine.

Chromatographic Constants - Interest is developing[1,48] in the use of R_M values, obtained from paper or thin layer chromatography, to serve as a measure of hydrophobic character. Some evidence has been found[1] to show a linear relation between π and ΔR_M. Further evidence from the work of Bark and Graham[35] is contained in eq 25. In eq 25, R_M values were ob-

$$R_M = 0.596\pi - 0.511 \qquad n = 47 \quad r = 0.938 \quad (25)$$

tained from reversed phase thin layer chromatography using cellulose impregnated with ethyl oleate as the stationary phase. Aqueous ethanol (25% v/v) was used as the mobile phase. The π values are from the phenol system.[15] Considering the movement of drugs through tissue as a kind of chromatographic process can lead to new insight in structure-activity study. For example, it is well known that stereoisomers move at different rates in chromatography. Even optical isomers can be separated when one employs an asymmetric adsorbent. This fact must be taken into account when one attempts to rationalize the difference in biological activity of different stereoisomers. At present, the almost universal tendency is to assume that differences in activity mean differences in fit to an asymmetric receptor site. A most important observation by Portoghese, Mikhail and Kupferberg[50] shows that the difference in brain concentration of epimeric analogs of meperidine is quantitatively related to their partition coefficients. This observation may well explain many examples that have been found where small to large differences in activity are recorded for different stereoisomers.

M.O. Parameters - Progress continues to be made in the correlation of pharmacological activity with electronic indices calculated from the molecular orbital approximation of quantum chemistry.[36-39] Purcell and Singer[40] have reviewed the parameters of use in the Hückel method. However, as has been previously noted,[1] it is rarely observed that quantitative equations such as those shown in this review result considering only M.O. parameters. Neely's work[38] does show that under certain limiting conditions such correlations can be found. The findings of Foernzler and Martin[37] are more typical. Although our understanding of free-energy related extrathermodynamic relationships even in homogeneous systems is still far from complete[52,53] applications of important practical as well as theoretical value are being made in the area of medicinal chemistry.

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Chapter 34. Alkaloids

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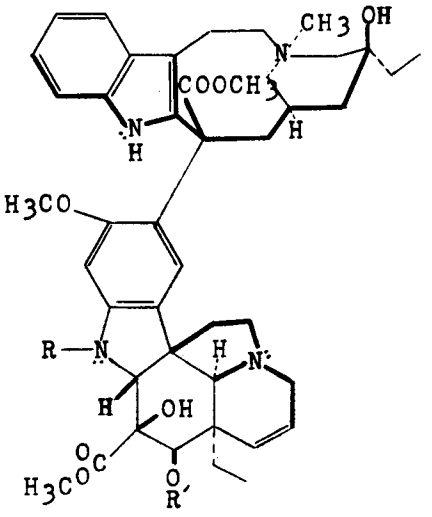
Introduction - As the search for new pharmacological prototypes continues, phytochemical screening programs and research efforts continue unabated throughout the world. Most of the screening effort has been directed toward the discovery of effective antitumor agents and, as a result, more potentially useful compounds have been discovered in this area than in any other. A good deal of research effort has been directed toward the proof of structure, as well as in synthetic efforts, of those novel compounds in order to more fully understand the biochemistry involved.

This review will be limited essentially to a discussion of those alkaloids possessing clinical activity *per se* or which appear to be potentially useful.

Symposia - Five symposia relating specifically to this area have been held during the past several years. Chemistry and biological manifestations of the alkaloids of *Catharanthus roseus* (L.) G. Don and other related plants of the family *Apocynaceae* have been published in full.[1] Two symposia,[2,3] specifically dealing with the biological functions and clinical activities of the oncolytic alkaloids of *C. roseus*, vincaleukoblastine (VLB, vinblastine) and leurocristine (vincristine), have been published in full. Two other symposia dealing with the general aspects of the search for antitumor agents of plant origin have been held, one having been summarized,[4] the other having been published in full.[5]

Catharanthus roseus (L.) G. Don - Structures for the two clinically active oncolytic alkaloids, vincaleukoblastine (VLB) (I, R=CH_3, R′=$COCH_3$) and leurocristine (LC) (II, R=CHO; R′=$COCH_3$) have been elucidated, these having been accomplished by utilization of a combination of chemical and physical techniques.[6] The complete molecular structure, including the stereochemistry and the absolute configuration of leurocristine methiodide dihydrate, has been determined by the combination of two crystallographic methods based on the anomalous scattering of X rays. The structures of both leurocristine and VLB have therefore been established.



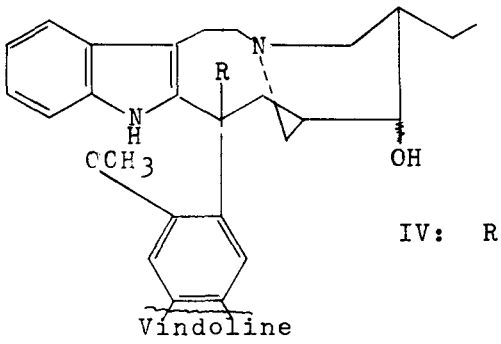
I: R=CH_3, R'=$COCH_3$

II: R=CHO, R'=$COCH_3$

III: R=CH_3, R'=$COCH_2N(CH_3)_2$

The elucidation of the structures of VLB and leurocristine allows for a rational and systematic approach to their synthesis, as well as for a logical pursuit of structure-activity relationships. In a series of α-aminoacetyl analogs,[8] VLB-4(N,N-dimethylaminoacetate) (III, R=CH_3, R'=$COCH_2N(CH_3)_2$) displayed excellent experimental oncolytic activity[9] and was selected for clinical testing. While initial results appeared to be favorable,[10] certain toxic manifestations involving serious eye problems (corneal and lens changes) were observed in two patients on long term therapy. Although causal relationship was not definitely documented, clinical trial with this compound has been discontinued.[11]

Until just recently, the structure of the profoundly active (experimentally) oncolytic alkaloid leurosidine has eluded elucidation. Spectral data (UV, IR and NMR), functional group determination, elemental analysis and mass spectral data for the parent alkaloid and its derivatives agree with a formulation of $C_{46}H_{58}N_4O_9$, isomeric with VLB. The isolation of desacetylvindoline has established the identity of the dihydroindole portion of the leurosidine moiety, this being the same as in VLB. The difference between the two alkaloids therefore resides in the indole portion of the molecule. Subsequent cleavage reactions and studies of the fragmentation patterns have allowed for the postulation of a structure for leurosidine[12] in which the hydroxyl at C-3' is probably α-oriented (IV, R=$COOCH_3$).



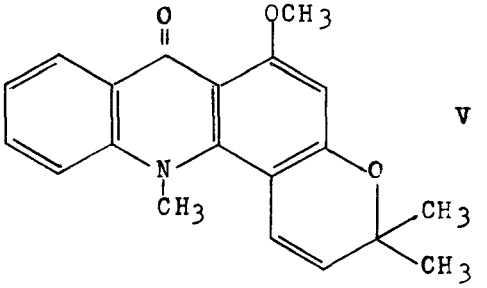
IV: R=COOCH3

Vindoline

The fourth oncolytic alkaloid, leurosine, $C_{46}H_{56}N_4O_9$, still resists complete structural elucidation. A partial structure has been proposed[13] but is at variance with the epoxide structure of Abraham and Farnsworth.[14]

The monoterpenoid origin of the indole alkaloids has now been established. While the C_{9-10} unit of the indole alkaloids is of mevalonoid origin, geraniol is a precursor of representative examples of the Corynanthe, Iboga and Aspidosperma groups of bases which collectively account for the majority of indole alkaloids.[15-25]

Acronychia Baueri Schott - The most interesting alkaloid isolated to date from the Australian scrub ash is acronycine.[26,27] While this acridone base (V)[28] has been found to possess the broadest antitumor spectrum of any known alkaloid, its clinical activity remains to be determined.

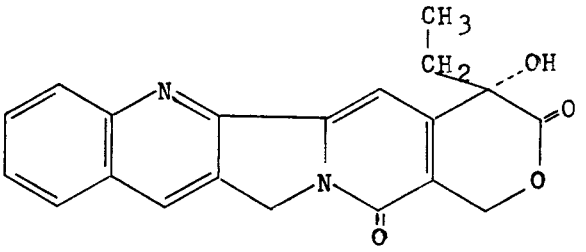


V

The experimental systems of special interest which are responsive to this alkaloid are: X-5563 plasma cell myeloma, a model system of multiple myeloma in man; Shionogi carcinoma 115, an androgen-dependent tumor, potentially a model system for prostatic cancer; the C-1498 myelogenous leukemia which is nonresponsive to any of the clinically useful chemotherapeutic agents.

The total synthesis of this natural product via two different routes[29] confirms the above angular structure. A third method has been successfully designed[30] and full details will be published.

Camptotheca acuminata Decne - This monotypic genus, native to the mainland of China, has yielded camptothecin(e), an alkaloid (VI) possessing a novel ring system.[31]



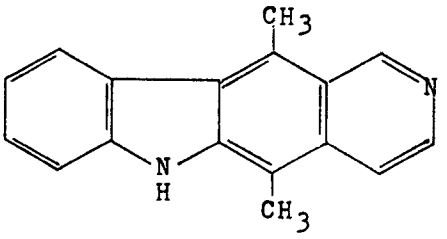
VI

Camptothecin(e) is active against the L-1210 leukemia and the Walker 256 carcinosarcoma, two experimental neoplasms which serve as the primary in vivo screen for testing in the program of the National Cancer Institute. This alkaloid is a likely candidate for clinical testing.

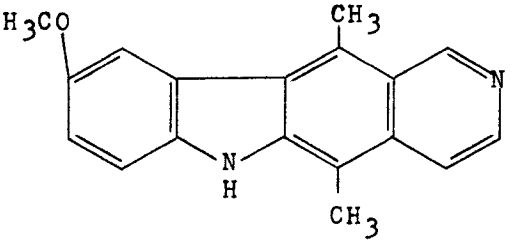
Yields from the natural source are reportedly quite low and the synthesis of the parent compound and simpler analogs is being actively pursued, as are the effects of structural modification on biological activity. While no announcement of its synthesis has yet been made, a model compound has been prepared.[32]

Ochrosia and Excavatia sp. - A considerable amount of work has been directed toward other plants of the Apocynaceae family and two of the more promising alkaloids isolated from several species are ellipticine (VII) and 9-methoxyellipticine

(VIII).[33,34]



VII



VIII

Both alkaloids have been shown to possess experimental oncolytic activity against the L-1210 leukemia, sarcoma 180 and adenocarcinoma 755.[35] In our laboratory 9-methoxy-ellipticine has shown activity against 10 of 17 mouse neoplasms tested, being active via both the intraperitoneal and oral routes of administration. While it does exhibit a broader spectrum than most available clinically active compounds, its activity is of a somewhat lesser order of magnitude. Toxicity effects have been manifested by weight losses, in some instances these being very severe, and this may well affect its utility as a clinically acceptable agent.

The yields of these alkaloids from the natural sources are not large enough to allow for extended clinical testing. Consequently, synthetic approaches have been devised to provide the required quantities needed. Both ellipticine and 9-methoxyellipticine were obtained in satisfactory yields, the latter having thus been synthesized for the first time, thereby affording a rigorous proof of structure.[35] Orthophosphoric acid was used to accomplish the cyclization of azomethines of 3-formylcarbazoles and aminoacetyl to substituted pyrido [4,3-b] carbazoles according to the scheme proposed by Cranwell and Saxton.[36] This appears to be the first reported use of orthophosphoric acid to effect this reaction.

Miscellaneous - A number of unusual dimeric indole alkaloids have been studied and structures have been postulated primarily on the basis of interpretation of physical data. The biological activities of these entities are presently unreported. Pleicarpa pycnantha has yielded one such new

dimeric, $C_{40}H_{46}N_4O_2$.[37] The chemistry of vobtusine, an alkaloid obtained from various Voacanga and Callichilia species has been defined.[38]

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SUBJECT INDEX

Listings are usually by generic name if available.